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ETIOLOGY AND CONTROL OF SNOW MOLD OF TURF IN THE PACIFIC NORTHWEST[1]

Jack P. Meiners

SUMMARY

Snow mold of turf in the Pacific Northwest is associated with either or both of 2 organisms: *Fusarium nivale* and *Typhula itoana*. Epidemiology depends upon certain weather conditions and varies with the particular organism involved. The latter species incites disease development only when the turf is snow covered. In contrast, the disease associated with the former species develops not only under snow cover but also in the absence of snow during cool, moist periods in fall and spring. Damage to susceptible turf areas may be severe, and control measures must be provided to maintain healthy turf.

The results of 3 years of testing a number of fungicides for snow mold control on golf greens in the area indicate that application of phenyl mercury formulations (PMAS, Phenyl Mercury Acetate Solubilized No. 10, Puraturf, and Tact-C-Lect) consistently provided the greatest reduction in incidence of the disease. These were followed in order of decreasing control by a mercury chloride mixture (Calo-clor), and cadmium succinate (Cadminate). Thiram (Tersan) and chloranil (Spergon) were inconsistent in controlling the disease. The remaining fungicides tested (Calocure, Puraturf GG, Crag 531, C. & C. 1025, Special Semesan, Puraturf 177, Orthocide 406, and Actidione) did not provide adequate control.

Snow mold of turf, associated with *Fusarium nivale* (Fr.) Ces. [*Calonectria graminicola* (Berk. & Br.) Wr.] and *Typhula itoana* Imai, is the most destructive disease of turf in many parts of the Pacific Northwest. It occurs most consistently in parts of Eastern Washington, Northern Idaho, and Western Montana where snow covers the turf for long periods. The disease is especially widespread on species of bentgrasses commonly used for fine turfs, such as those on golf courses, in parks, in cemeteries, and in home lawns. Golf greens are made up almost exclusively of bentgrasses, and for this reason snow mold is an important problem in greens maintenance.

Attempts to control snow mold on turf in this region by the application of recommended fungicides have been only partially successful. Furthermore, these fungicides are expensive, both in cost of material and their application, and under some conditions they are injurious to the turf. Tests were undertaken to determine whether, among certain newly developed chemical preparations, there might be any that would provide better and more economical control of snow mold. These tests were made in connection with the National Cooperative Turf Fungicide Trials of the American Phytopathological Society and the Pacific Northwest Turfgrass Association.

In addition to the fungicide tests, observations were made on the causal organisms associated with the disease and on the seasonal development in the area. This is a report on these tests and observations.

LITERATURE REVIEW.—Several fungi have been reported to be associated with snow mold of turf in the United States and Canada (3, 5, 6, 17, 19). In early studies of this disease *Fusarium nivale* was the primary species considered (5, 9). Later, *Typhula itoana* was found to be associated with a snow mold type of disease (17, 19). The disease associated with the former species has been called pink snow mold to distinguish it from the Typhula type, which has been designated as snow scald (7, 13). Sprague (15) has reported both species on cereals and on forage and range grasses in Washington. Blodgett (2) has made similar observations in Idaho.

Control of Fusarium snow mold of turf by fungicidal treatments was first reported by Monteith (9), who used corrosive sublimate. Since that time, numerous materials, primarily mercurials, have been found to give fair to good control (1, 5, 18). The relative effectiveness of these materials apparently is related to the mercury content (18). Typhula snow mold of turf has also been controlled with varying success by mercury and certain other treatments (11, 20).

At the present time, the standard treatment for the prevention and control of snow mold of turf is the application of mercury chlorides (Calomel-corrosive sublimate mixture) at the rate of 2-4 oz. per 1000 sq. ft. in late autumn and during mid-winter thaws (7, 10).

MATERIALS AND METHODS.—The fungicide tests for snow mold control were conducted on golf greens at Spokane and Pullman, Washington. At both locations, the greens were seeded initially to seaside bent (*Agrostis palustris* L.) but annual bluegrass (*Poa annua* L.) has become an important constituent of the turf. The greens were last fertilized in August, using a nitrogen fertilizer at about 2 lb. actual N per 1000 sq. ft. Most of the tests were conducted on replicated plots 5 × 10 ft. in size, but plots 8 × 80 ft. in size were used at Pullman in 1952-53. The fungicides were applied either dry, using sand as a carrier at the rate of 10 quarts per 1000 sq. ft., or as a spray, with water as a diluent at the rate of 5 gal. per 1000 sq. ft. In the lat-

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ter case, nozzle pressure was approximately 30 lb. per sq. in. The materials were applied in late autumn just before the first snow fall. The percentage of diseased turf was recorded the following spring, 1–2 weeks after the turf was free of snow.

The following fungicides were tested: Acti-dione (β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide), Crag 531 (Copper cadmium zinc calcium chromate complex), C & C 1025 (copper mercury cadmium zinc calcium chromate complex 2.5 CuO · 0.15 HgO · 6CdO · Zn 0.10 CaO · CrO_3), Calo-chlor (60 per cent mercurous chloride and 30 per cent mercuric chloride), Calocure (30 per cent mercurous chloride and 15 per cent mercuric chloride), Cadminate (60 per cent cadmium succinate), PhenylMercury Acetate Solubilized No. 10, Puraturf (6 per cent phenylmercurytriethanolammonium lactate), Puraturf 177 (*p*-aminophenylcadmium dilactate), Puraturf GG (Organic mercury cadmium complex), PMAS (10 per cent phenylmercury acetate), Special Semesan (24 per cent hydroxymercurichlorophenol, 3.9 per cent hydroxymercuricresol), Tersan which is 75 per cent thiram (bis(dimethylthiocarbamoyl) disulfide), Spergon which is 48 per cent chloranil (tetrachloro-*p*-benzoquinone), Tact-C-Lect (4.75 per cent phenylmercury acetate), Orthocide which is captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide).

ETIOLOGY AND DEVELOPMENT.—Observations on cause and seasonal occurrence of snow mold were made over a 3-year period on golf courses in eastern Washington and northern Idaho. Both types of new mold were found throughout this area. In the majority of cases the Fusarium type was predominant not only on the greens but on the fairways as well. In many cases, only the Fusarium type of snow mold was present. In a few cases, the Typhula type was predominant. The predominance of one or the other type followed no regular pattern and varied from year to year. Since these 2 types of snow mold and their causal agents occur together in the area more often than not, it seems impractical to consider them as distinct diseases for control purposes. Thus, snow mold is used here as an inclusive term, embracing both types.

The general symptoms of snow mold on turf in this area agree with those reported from other areas (5, 7, 19).

The general epidemiology of the 2 types of snow mold in this region apparently is related to certain weather factors. For Fusarium snow mold of turf, this relationship is similar to that observed in other areas of infestation (4, 5, 9, 14). First symptoms of the pink snow mold appear on golf greens in October to November, coincident with cool moist weather conditions, and usually long before the turf becomes dormant or snow falls. The disease is first noticeable as circular areas 3–5 cm. in diameter. At this time, the mycelium of the fungus is readily visible in the diseased areas. Development of the disease may continue all winter under cover of snow and into the spring after the snow melts and as long as cool moist conditions prevail, usually throughout February and March in this area.

In contrast to Fusarium snow mold, symptoms of the disease associated with Typhula do not appear in the fall prior to snow fall but are evident only after the snow melts in early spring. However, beginning in late October and continuing until mid-December, sporophores are produced from germinating sclerotia that have been dormant throughout the summer months. These sporophores are typical of those of *Typhula itoana* (12) and may be so numerous that patches of the turf may appear pink in color. These areas are irregular to circular in shape and vary from 6 in. to several feet in diameter. It was observed that the areas where sporophores were produced corresponded to snow mold spots of the previous spring. A similar relationship was noted by Tervet (17) in Minnesota.

Basidiospores are produced in abundance on these sporophores, but whether they play any part in producing snow mold of turf is not known. This would seem likely since Sprague (16) has shown that basidiospores discharged from sporophores of *T. itoana* and *T. idahoensis* in late fall cause infection of winter wheat. Even though infection may be initiated in late fall, the disease develops only under snow cover and symptoms do not become apparent until the following spring.

Under favorable conditions, and especially when snow falls on turf before the ground is frozen, snow mold may attack large areas of susceptible turf such as a golf green or lawn. However, in eastern Washington and adjacent areas, usually only the leaves and stolons of the grass are killed and the turf recovers. This recovery is very slow and consequently snow-molded areas are visible into May and June. Adequate control is imperative, therefore, if good turf is to be maintained.

RESULTS OF FUNGICIDE TESTS.—Fungicide tests were

TABLE 1.—*The effect of 15 fungicides on the percentage of snow mold at Pullman and Spokane, Washington in 1951-52*

Treatment	Dosage per 1000 sq. ft.	Percentage Snow mold[a] Pullman	Spokane
Untreated	———	95.6	18.1
Actidione	200 ppm	96.9	18.2
Crag 531	3.0 oz.	87.1	22.2
C & C 1025	3.0 oz.	89.5	18.7
Calo-clor	2.0 oz.	38.2	5.0
Calocure	2.0 oz.	74.5	9.5
Cadminate	1.6 oz.	57.1	8.7
Puraturf	0.2 pts.	24.5	1.2
Puraturf 177	1.6 oz.	91.1	16.2
Puraturf GG	0.05 pts.	83.1	5.2
PMAS	0.1 pts.	23.1	1.5
Special Semesan	3.0 oz.	89.6	11.2
Tersan	3.0 oz.	95.9	3.0
Spergon	3.0 oz.	95.9	4.0
Tact-C-Lect	1.5 oz.	76.2	6.5
Orthocide 406	3.0 oz.	92.2	9.5

[a] Pullman—average of 4 replications; Spokane—average of 2 replications.

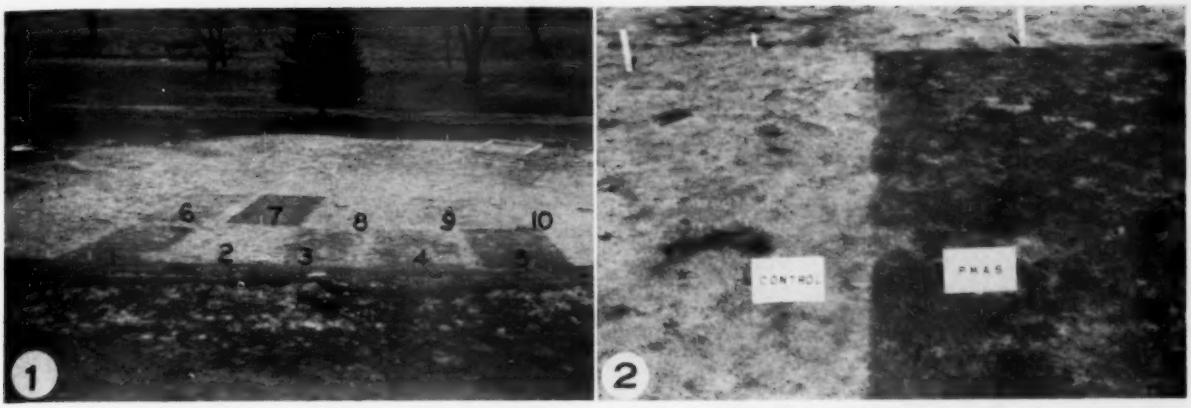

FIG. 1–2. Fig. 1. View of the plots on one of the greens at Pullman, showing degree of infestation and the amount of control obtained with some of the treatments in 1951-52. Treatments in the foreground are: 1) Calo-clor, 2) Untreated, 3) Calocure, 4) Cadminate, 5) Puraturf, 6) Puraturf GG, 7) PMAS, 8) Untreated, 9) Special Semesan, 10) Tersan. —Fig. 2. Closeup of 2 of the plots showing the PMAS treated plot with the untreated plot adjacent to it.

conducted over a 3-year period embracing the winter seasons of 1951–52, 1952–53 and 1953–54. The winter of 1951–52 was very favorable for development of snow mold, particularly at Pullman (Fig. 1–2), where the greens were covered with snow from early December until mid-March and the ground beneath the turf remained unfrozen. *Fusarium nivale* was the dominant organism at Pullman, whereas *Typhula itoana* was more apparent at Spokane. Results of the 1951–52 tests are summarized in Table 1. A preliminary report on the results of these tests has been published previously (8).

Under conditions of severe infestation, and light dosages, none of the materials provided efficient control at Pullman in 1951–52, whereas under conditions of mild infestation at Spokane, some materials provided adequate control. Two phenyl mercury compounds (Puraturf and PMAS) provided the greatest reduction of snow mold at both locations, followed closely by the mercury chloride compound, Calo-clor. Application of Tersan, Spergon and Puraturf GG also resulted in low percentages of snow mold at Spokane, but gave little or no control at Pullman. The remaining fungicides were inferior to those mentioned in reducing the incidence of the disease.

In an additional experiment at Pullman in 1951, methods of application (wet vs. dry) were compared using 9 of the 15 materials listed in Table 1. Results obtained in this test indicated that method of application used had no effect on degree of control except in the case of Cadminate where the dry treatment resulted in less snow mold than did the wet treatment. For this material percentages of snow mold were 49.7 and 62.2, respectively.

In spite of a mild winter with little snow cover, abundant snow mold developed on untreated plots in both locations in 1952–53. At Pullman, the disease again was associated primarily with *Fusarium nivale*, and at Spokane, *Typhula itoana* was again the dominant organism.

The results obtained in 1952–53 (Table 2) are similar to those obtained the previous year. The liquid phenyl mercuries (PMAS, Puraturf, Phenyl Mercury Acetate Solubilized No. 10, Tact-C-Lect) were outstanding in reducing the percentage of snow mold. Cadminate, used at much heavier dosages than pre-

TABLE 2.—*Effect of fungicides on percentage snow mold at Pullman and Spokane, Washington, in 1952–53*

Treatment	Dosage per 1000 sq. ft.	Percentage Snow Mold[a]
Pullman		
Untreated	——	48.0
Phenyl Mercury Acetate Solubilized No. 10	2 oz.	1.7
Tact-C-Lect 10%	2 oz.	1.7
PMAS 10%	2 oz.	1.8
Puraturf 6%	3 oz.	2.0
Cadminate	2 oz.	3.8
Calo-clor	3 oz.	4.8
Special Semesan	5 oz.	9.6
Puraturf GG	1 oz.	10.6
Calocure	3 oz.	17.5
Spergon	6 oz.	35.5
Tersan 75	6 oz.	38.0
Spokane		
Untreated	——	26.7
Cadminate	2 oz.	0.0
Cadminate	4 oz.	0.0
Phenyl Mercury Acetate Solubilized No. 10	0.1 pt.	1.5
Phenyl Mercury Acetate Solubilized No. 10	0.2 pt.	0.0
PMAS 10%	0.1 pt.	3.5
PMAS 10%	0.2 pt.	0.0
Tersan 75	3 oz.	4.0
Tersan 75	6 oz.	1.5
Calo-clor	2 oz.	5.7
Calo-clor	3 oz.	4.0
Calocure	3 oz.	6.2
Calocure	4 oz.	4.7
Spergon	3 oz.	2.2
Spergon	6 oz.	6.7
Special Semesan	3 oz.	17.5
Special Semesan	6 oz.	9.7

[a] Pullman—average of 3 replications; Spokane—average of 2 replications.

viously, also gave excellent control at Spokane although some temporary injury to the turf was evident at the heavier dosage. This same material ranked just behind the phenyl mercuries at Pullman.

Calo-clor also reduced snow mold percentages considerably at both locations but ranked well behind the phenyl mercuries in giving efficient and consistent control of the disease. As in 1951–52, Tersan was effective at Spokane, but ineffective at Pullman. The remaining fungicides tried did not provide adequate control of the disease at either location.

An additional trial was conducted on golf greens at Pullman to determine the minimum effective dosage of PMAS required to control snow mold. It was found that 1 oz. in 5 gal. of water applied to 1000 sq. ft. of turf did not provide so good control as did 2 oz. but that 3 oz. provided no additional protection. Where ½ gal. of water per 1000 sq. ft. was substituted for 5 gal. as a diluent, no difference was noted in degree of control obtained.

In 1953–54, the fungicides that had shown best control in the 2 previous years were compared, using larger plots and employing the equipment and methods ordinarily used by greenskeepers to apply the materials. Adequate control was obtained with application of PMAS, Cadminate, and Calo-clor, but no reduction in incidence of snow mold was obtained using Tersan. The dominant organism was *Fusarium nivale*. It was also found that 2 oz. of PMAS applied in mid-November gave adequate control, whereas an application of 3 oz. of PMAS applied in December during the first snowfall was too late to prevent an earlier infestation.

Discussion.—The data presented here indicate that phenyl mercury fungicides are superior to previously recommended fungicides, such as the mercury chlorides, in reducing snow mold infestation on golf greens in the Pacific Northwest. In addition, the former are less costly and, according to reports from greenskeepers, are easier and less expensive to apply than fungicides formerly in common use. Therefore, many greenskeepers in the area are applying these fungicides to tees and other susceptible turf areas as well as to the greens themselves. It may be noted also from these tests that degree of control does not appear to be related to the mercury content of the fungicide, as has been reported previously (18), since the phenyl mercuries have a lower mercury content than many of the fungicides providing less control.

Although the mercurials as a group were superior to the non-mercurials in reducing snow mold infestation, one cadmium compound (Cadminate) appears to be promising. Since Cadminate also provides excellent control of dollar spot, it might, therefore, be used as a dual purpose fungicide to control a late infestation of this disease and, at the same time, prevent an early infestation of snow mold.

Thiram (Tersan) and chloranil (Spergon) have been shown to be inconsistent in reducing the incidence of the disease in the Northwest. These inconsistencies may be related to the fact that the disease may be associated with either or both of the 2 parasitic fungi. It has been noted that the materials in question provide adequate control only when *Typhula itoana* is the sole or dominant pathogen. Thus these fungicides appear to be more specific in their action.

Washington Agricultural Experiment Stations
Pullman, Washington

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STUDIES ON SEED TRANSMISSION OF LETTUCE MOSAIC VIRUS [1, 2]

Houston B. Couch [3]

SUMMARY

Individual plants of the variety Bibb vary considerably in ability to transmit common lettuce mosaic virus through seed. Percentage of seed transmission of the virus in the progeny of 3 plants was different from that of the parents, but the rate in 3 generations of progeny from a fourth plant was essentially the same as that of the parent.

The inflorescences of 2 lettuce plants, infected through seed, were mapped with respect to position and time of floral initiation and the seed from the different positions on the seed stalk checked for percentage of seed transmission. Rate of seed transmission was distributed among the various floral heads in a randomized manner irrespective of topographical relationships or time and order of floral initiation.

Lettuce plants, inoculated just before flowering, produced fewer virus infected seed than those inoculated soon after planting. Plants that became infected after flowering had started did not transmit the virus through seed.

The reported absence of seed transmission of the virus in the variety Cheshunt Early Giant was confirmed. The first-formed floral heads of infected plants of this variety were killed by the virus; however, these plants recovered and produced normal seed. Juice from recovered shoots produced half as many local lesions on *Gomphrena globosa* L. as did juice from the vegetative tissue of younger diseased plants.

In Cheshunt Early Giant, the procambial and epidermal tissues of the peduncle were especially sensitive to the virus. Complete obliteration of the procambial tissue was characteristic whereas the vessels and laticiferous elements were filled with a gummous exudate.

Seed transmission of lettuce mosaic virus was first reported by Newhall (14) in 1923. Not only has this observation been confirmed by later workers, but it has also been shown that infected seed constitute the most important source of primary inoculum. The importance of the seed-borne nature of the virus has been given even more emphasis with the recent report of Grogan et al. (5) that the disease can be effectively controlled in California by the use of virus-free seed.

The purpose of this investigation was to examine certain aspects of seed transmission of lettuce mosaic virus in an attempt to arrive at a better understanding of some of the factors that influence this phenomenon.

FACTORS INFLUENCING PERCENTAGE OF SEED TRANSMISSION.—*Variation among individual plants of a given variety.*—It has been reported that in both soybean and garden bean there is variation among different varieties, and individual plants within given varieties, for seed transmission of virus (3, 12, 13, 18). That varieties of lettuce vary in ability to transmit lettuce mosaic virus through seed has been illustrated by Kassanis (11), Grogan et al. (5), Fry (4), and Ogilvie and Brian (15). Since variation exists among varieties of lettuce with respect to percentage of seed transmission, there is a possibility that the propensity for seed transmission of the virus is governed by differences in genetic constitution. Selection within a given variety for non-seed transmission might, therefore, be the most desirable solution of the problem of obtaining virus-free seed stocks.

Grogan et al. (5) have reported that mosaic-diseased plants of the variety Bibb transmit the virus through 8 per cent of their seed. Because of this comparatively high rate of seed transmission, this variety was chosen for investigation.

Seed of each of 4 seed-borne mosaic plants was harvested separately and checked for rate of virus transmission. This determination was made by planting all the seed produced by each parent plant and counting the seedlings for seed-borne infection when they were 6 weeks old. The percentages of seed transmission of these 4 plants (A, B, C, D) were as follows: A) 11.31 per cent; B) 4.03 per cent; C) 7.02 per cent; and D) 10.03 per cent.

Ten plants infected through seed were saved from each of these 4 parent lines. The seed produced by each of these plant groups was bulked and enough planted from each lot to give a seedling count of at least 1,000 plants from each parent line. This F_1 count revealed a deviation of approximately 2–3 per cent from the parents for lines A, B, and D; line C varied only 0.15 per cent from its parent's rate of transmission. A similar count was made on the F_2 generation of lines B and C, which showed that group B varied 2 per cent from its parent and line C deviated only 0.35 per cent from its parent.

Four seed-borne mosaic F_3 plants of each of lines B and C and F_2 plants of line A were selected at random. Seed from the individual plants was harvested separately and planted and a count for seed-borne mosaic virus transmission was made when the progeny were 6 weeks old. This count included all the seed that each plant produced. A total variation of 3 per

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cent among the 4 plants, and a deviation of almost 6 per cent from the original parent count, was found for line A. For line B a total variation of 10 per cent among the 4 plants, and a spread of almost 3 per cent below and 7 per cent above the original parent count was found. A total variation of only 0.5 per cent among the 3 progeny and a spread of 0.6 per cent from the original parent count was found for line C. This is based on a count of the seed from only 3 parent plants of line C because the seed of the fourth failed to germinate in sufficient numbers to give an adequate measurement of the rate of transmission of the virus.

Thirty additional seed-borne, mosaic-diseased plants were selected at random from a commercial stock. All the seed from each of these plants was harvested and planted separately. The percentage of seed transmission varied from 0.21 per cent to 14.18 per cent among the individual plants, with the greatest number of plants falling within the 6-12 per cent range. A total of 37,904 plants were counted, with 2,988 of these showing mosaic symptoms. This gave an average of 7.88 per cent seed transmission, which apparently is not significantly different from the 8 per cent reported by Grogan et al. (5).

Time and order of floral formation.—Kendrick and Gardner (12), working with soybean mosaic, found no correlation between the percentage of seed-borne virus and location of the seeds on the plants. Fajardo (3) and Nelson (13) also stated that there was no uniformity in distribution of bean mosaic virus to the seeds in various pods on individual plants or to the seeds in each pod. Harrison (6), on the other hand, reported that seeds in early formed pods on seed-borne infected plants transmitted bean mosaic virus more frequently than seeds from pods formed later in the flowering period. To determine the distribution of seed-transmission of lettuce mosaic virus within the inflorescences of mosaic-diseased lettuce plants, 2 such plants of the variety Bibb were indexed on the basis of (a) location of the floral heads and (b) time and order of floral formation. The seed was harvested and planted in the separate groups and a count for percentage of infection made when the plants were 6 weeks old. This count showed the percentage of seed transmission to be distributed throughout the inflorescences of the 2 plants in a randomized manner irrespective of topographical relationships or time and order of floral formation. The degree of seed transmissible infection of single floral heads was found to range from 0 per cent to 35 per cent.

Time of infection of parent plant.—Both Fajardo (3) and Harrison (6) have reported a correlation between the age at which bean plants become infected and the percentage of seed transmission of bean mosaic virus. Kendrick and Gardner (12), however, stated that the variation in percentage of seed transmission of soybean mosaic virus was not dependent upon the date of infection of the parent plant.

To determine whether a correlation exists between time of infection and percentage of seed transmission of lettuce mosaic virus, 5 groups of plants of the variety Bibb representing different growth stages were established. The first group was infected through seed whereas the last 4 groups were inoculated by the use of viruliferous green peach aphids, *Myzus persicae* Sulz., at the following ages: 1) 4 weeks, 2) 9 weeks, 3) 13 weeks (just prior to bolting), and 4) 21 weeks (the plants were flowering and ⅓ of the seed was set). To avoid individual plant variation, each group index was based on bulked seeds of 7 infected plants. A count of at least 1,000 progeny from the seed of each group revealed a decreasing percentage of seed transmission as plants were infected closer to the time of flowering. The group infected through seed and the group inoculated when 4 weeks old were the 2 highest transmitting, their rates being 7.42 per cent and 7.89 per cent, respectively. The group inoculated after flowering did not contain any mosaic-diseased seedlings in those counted whereas the intermediate groups, 9 and 13 weeks, fell between these 2 extremes with transmission rates of 4.91 per cent and 4.63 per cent.

In another test, the seed plants were inoculated soon after the first evidence of axis elongation and subsequent separation of the terminal cluster group of floral heads. That infection of these seed plants had been accomplished was proved by inoculating *Gomphrena globosa* L., a local lesion host recently described by Wilkinson and Hirsch (19), with expressed sap from each plant of this group. None of the 972 seedlings counted from the seed of these plants showed any evidence of mosaic.

RELATIONSHIP BETWEEN VIRUS ACTIVITY AND FLORAL DEVELOPMENT IN THE VARIETY CHESHUNT EARLY GIANT.—In reports based on the absence of seed-borne, mosaic-diseased plants in the field, Kassanis (11) and Broadbent (2) stated that the variety Cheshunt Early Giant did not transmit lettuce mosaic virus through seed. It was felt that before this point could be established as factual, it would be necessary to test seed from known mosaic-diseased parent plants.

Seed was harvested from 60 field-grown, mosaic-diseased Cheshunt Early Giant plants that had been artificially inoculated with the virus when they were 6 weeks old, and positive identification of the disease made in each before transplanting. From this seed, 4,684 seedlings were checked for mosaic, and in no

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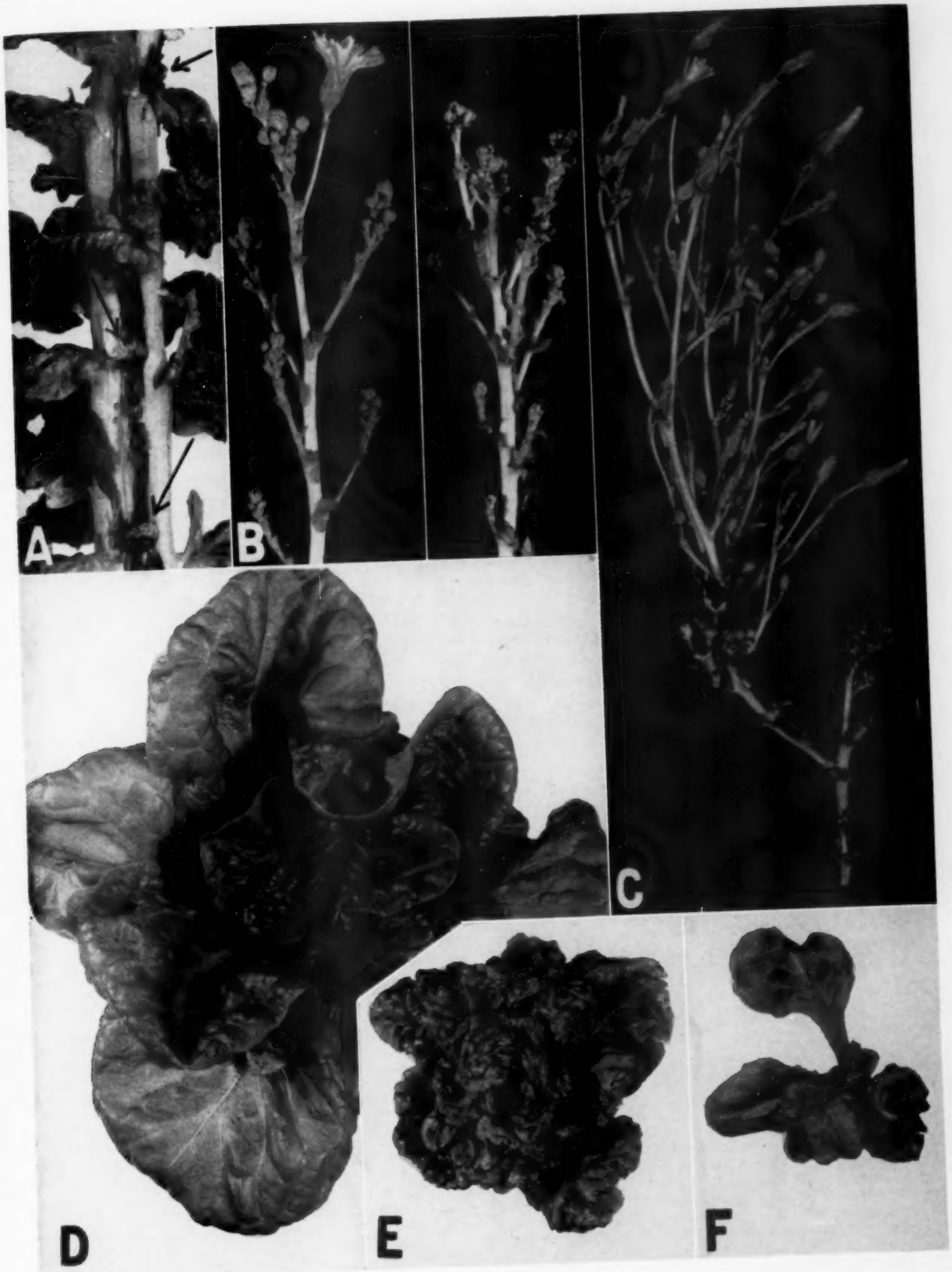
FIG. 1. Symptom expression of mosaic-diseased plants of the lettuce variety Cheshunt Early Giant. A) Stem of mosaic-diseased plant, showing necrotic streaking and tumor formation. B) Left, healthy floral heads; Right, floral heads from a mosaic-diseased plant of the same age. C) Production of normal side shoots after initial blighting. Note necrotic areas in the basal portion of the recovered side shoot group. D) Healthy 8-week old plant. E) Mosaic-diseased 8-week old plant, same magnification as D. F) Abnormal, but noninfected, seedling from a lot of seeds harvested from mosaic-diseased plants.


A
B
C
D
E
F

case was seed transmission of the virus observed. However, an abnormal condition of certain seedlings that could possibly be mistaken for mosaic was noticed. The plants were stunted and exhibited a considerable degree of leaf distortion in the first 2 or 3 leaves (Fig. 1, F). Plants of *Gomphrena globosa* were inoculated with juice from 4 of these abnormal plants; failure to obtain local lesions indicated that they were free of the virus. Furthermore, in all cases, recovery began with the production of the fourth true leaf, with ultimate complete recovery being accomplished.

The course of the disease in the variety Cheshunt Early Giant was characteristic. Infected plants were severely stunted. Leaves not only expressed a characteristic mosaic pattern but were also dwarfed, contorted, thickened, and brittle, and the margins frequently showed considerable necrosis (Fig. 1, D, E). When the plants bolted, large, dark-brown, necrotic areas developed on the seed stalks. Rifts occurred in these areas, and the profuse latex exudation that followed gave the stems a characteristic dark brown appearance. In many cases, tumors developed at the ruptured areas (Fig. 1, A). Necrotic areas formed on the developing involucral bracts and peduncles and all the first-formed floral heads were blighted before they were able to mature (Fig. 2, B). Eventually, however, new side shoots were produced from the axillary positions on the first-formed side branches. Floral heads that were formed on these new shoots produced normal flowers and set seed (Fig. 1, C). Even in these, however, the lower floral heads were often blighted before they were able to mature, and, occasionally, necrotic areas were observed on the bracts of the involucre of the uppermost heads. There are cases where similar host reactions, i.e. initial shock symptoms and then recovery, have been shown to be caused by varying levels of virus activity. Price (16) has shown, for example, that tobacco ring spot virus is 5 times more active in infected tobacco leaves than in leaves that no longer exhibit visible evidence of the disease. Furthermore, he illustrated that the virus was far more infective in parts of leaves that were showing ring spot symptoms than parts of the same leaves that had become symptomless. The following work was conducted to determine whether such a correlation exists between symptom expression and lettuce mosaic virus activity in the Cheshunt Early Giant variety.

The sources of inoculum were as follows: 1) recovered side shoots, including floral heads, peduncles, and leaflets; 2) necrotic side branches from the same plants as the former group; 3) necrotic tissue from plants that showed complete obliteration of the initial floral heads; 4) tissue from bolted plants in which the first-formed floral heads were just being initiated; and 5) tissue from 8-week old plants. All the source plants had been inoculated when they were 6 weeks old. In order to minimize the possibility of individual plant variation, 5 plants were used for each of the 5 types of inoculum. Plants of *Gomphrena globosa*, at the 6 true leaf stage of growth, were used as the local lesion host. The technique used for inoculation was similar to that described by Hirsch (8) as follows: The forefinger was dipped in the inoculum and then rubbed lightly over a leaf that had been evenly dusted with carborundum. Each leaf was rubbed 8 times, stroking always toward the tip, and after each sampling series, the hands were washed with soap and warm water. Because of the difficulty of standardization, chemical additives were not used with the inoculum. In order to randomize the variability caused by individual plant and leaf position factors of the local lesion host, the experiment was set up as a Latin square, with each Gomphrena plant representing a row. With this organization, each inoculation was replicated 25 times through a series of 5 blocks. The data obtained were subjected to analysis of variance.

The local lesion count showed the virus to be present in the recovered side shoots, but its activity was half that of the tissue from 8-week old plants. This difference in activity was significant at the 5 per cent level. These 2 growth stages represented the extremes in virus activity; the intermediate symptom expression groups appeared to be slightly, but not significantly, lower in infectivity than the 8-week old plants.

PATHOLOGICAL ANATOMY OF MOSAIC-DISEASED FLORAL HEADS OF THE VARIETY CHESHUNT EARLY GIANT.—Floral tissue from healthy and mosaic-diseased plants of the variety Cheshunt Early Giant was fixed and killed in Karpechenco's solution (17), dehydrated by the tertiary butyl alcohol technique described by Johansen (9), embedded in Fischer's Tissuemat, and sectioned. Conant's (9) quadruple stain schedule was used, with the following modifications: Safranin, 1½ hours; 1 per cent crystal violet, 1 minute; and a 1:1 absolute alcohol and xylene bath added between the second clove oil and final xylene baths.

Floral development and normal anatomy of the peduncle.—Jones (10) has studied the development of the floral heads of lettuce. He found that the meris-

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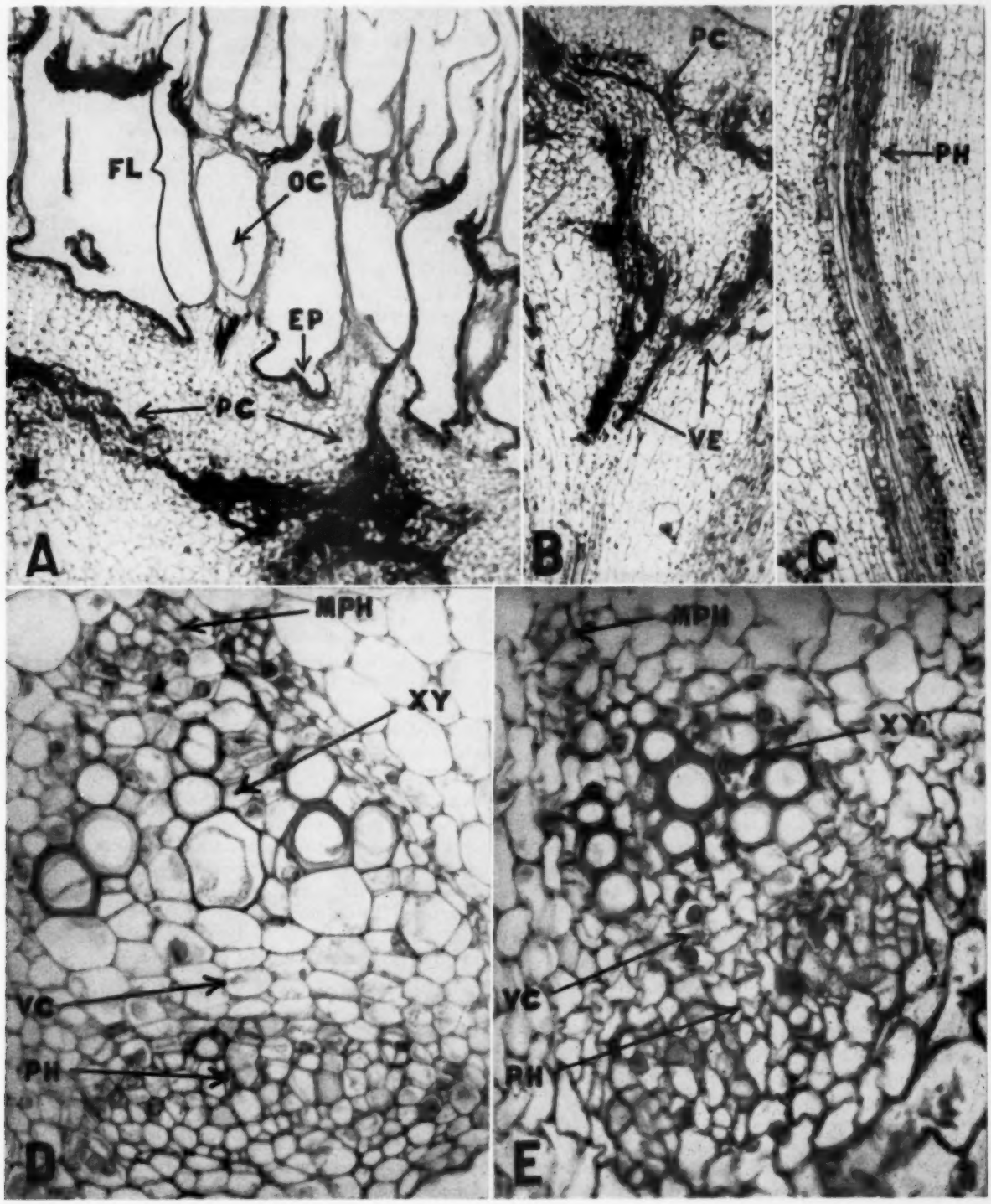
FIG. 2. Longitudinal and transverse sections of healthy and mosaic-diseased floral tissue of the variety Cheshunt Early Giant. A) Longitudinal section of a portion of a floral head, at ovarian cavity stage of growth, from mosaic-diseased plant. Note obliteration of procambial tissue leading up into the base of a floret, and necrotic epidermal cells. 100×. B) Longitudinal section of a portion of a receptacle. Note plugging of vessels by the gummous exudate, and obliteration of the procambial tissue. 95×. C) Longitudinal section of portion of peduncle near the second bract below the florets showing extensiveness of phloem degeneration. 95×. D) Transverse section of vascular bundle from the peduncle of a healthy plant. Taken from an area immediately below the receptacle. 606×. E) Transverse section of vascular bundle from the peduncle of a mosaic-diseased plant. Taken from same relative position as D. Note disorganization of the various tissues. 606×. EP, epidermis; FL, floret; OC, ovarian cavity; MPH, medullary phloem; PH, phloem; PC, procambium; VC, vascular cambium, VE, vessels; XY, xylem.

tematic region of the receptacle is at first convex, then becomes flattened and broadened, and gives rise to protuberances that develop into the individual flowers. These floral primordia are initiated simultaneously over the entire surface of the receptacle. As these primordia enlarge, a marginal ring occurs on each, marking the initiation of the corolla tube. Swellings appear inside the developing corolla tube, while simultaneously, at the base and outside the tube, an elevated ring of meristematic tissue is formed. The inner protuberances represent the initiation of the androecium and the outer ring is the developing pappus.



The carpels are the last organs to be differentiated and, by the time they appear, the corolla tube has started to curve inward over the tip of the floral axis. The ovarian cavity is then formed as a result of the upward growth of the carpellary tissue (Fig. 2, A). The ovule is initiated at the base of the ovarian cavity.

In this study, attention was given to the relation between stage of floral growth and development of the procambium. In the development of the receptacle of healthy lettuce plants, at both the conical and flattened stages of growth, visible evidence of differentiating procambium can be seen up to, and on a line with, the base of the developing bracts. Procambial differentiation up to the base of each floret occurs simultaneously with the formation of the floral protuberances. Procambial tissue then develops at a rate parallel with floral growth and, at the time of formation of the ovarian cavity, procambial formation is quite extensive throughout the receptacular region immediately below the florets. Just prior to the formation of the ovule, procambial strands can be seen leading up into the base of the florets and isolated strands of primary xylem are located on line with the base of the first bract below the florets.

The epidermis of the developing peduncle is uniserate. The cortex is 4–5 cells thick, composed of parenchymatous cells which vary considerably in size and are loosely arranged so that there are large intercellular spaces. Hayward (7) states that in the mature stem, the cortex is limited on its centripetal side by an endodermal layer; however, this layer is not evident in the developing peduncle and the cortical tissue is continuous through the interfascicular areas to the pith. The vascular system is comprised of an organized ring of collateral bundles, which are soon connected by interfascicular cambium, resulting in a continuous vascular cylinder in the mature stem. Secondary thickenings of the primary xylem of the peduncle at the ovarian cavity stage are spiral and the vessels are separated by narrow, elongated, parenchymatous cells (Fig. 2, D). Medullary phloem strands are formed in the pith opposite the protoxylem elements. This gives the appearance of bicollateral bundles. Hayward (7) points out, however, that these strands are late in forming and their arrangement and number adjacent to a bundle vary; therefore, they do not constitute an integral part of the vascular bundle (Fig. 2, D).

Pathological anatomy.—Blighting of the developing inflorescence appears to involve all the floral parts. The ovarian cavity stage of growth pictured in Fig. 2, A, represents the oldest stage reached by any of the floral heads before complete necrosis occurred. At this point, death of all other floral tissue, down through the conical stage of meristem development, is evident. Eventually the remaining parenchymatous cells of the receptacle and peduncle die and the entire shoot becomes blighted. The demarcation between the necrotic peduncle and stem is very distinct. The normal side shoots that develop later arise from meristematic tissue that forms after the obliteration of the first-formed floral heads.

Apparently the virus does not exert an initial retarding effect on procambial development, for that of mosaic-diseased plants appears to parallel the procambium of healthy floral heads in rate of growth up to the ovarian cavity stage of floral formation. However, at this stage there is a marked difference between procambium of diseased and healthy floral heads. The cytoplasm of the procambial cells of the mosaic-diseased floral heads becomes granular and finally agglutinates. The nuclei degenerate into amorphous masses that are indistinguishable from the red staining cytoplasmic material. Eventually, the entire procambium is obliterated (Fig. 2, A).

Breakdown of the parenchymatous cells of the receptacle appears to originate near the degenerating procambial region and to advance outward in all directions. The plastids and cytoplasm degenerate and become granular. The nuclei are at first more clearly defined in outline than those of healthy tissue. They then become more particulate than their healthy counterparts and degenerate into a granular mass which fuses with the now amorphous cytoplasmic contents.

Another focal point of cellular degeneration in the receptacular area is the developing epidermal layer (Fig. 2, A). Like the procambium, these cells appear to be particularly sensitive to the virus, and undergo a breakdown similar to that described for the procambial and adjacent parenchymatous cells. The parenchyma next to the degenerating epidermis is similarly affected and the process of cellular degeneration progresses inward from these areas of inception.

Necrotic areas commonly develop on the young bracts (Fig. 1, B). The necrosis may originate in 1 of 2 tissue groups—either in the epidermal cells proper, as described above for the receptacle, or in close proximity to the isolated strands of developing vascular tissue. Cellular degeneration then progresses outward from these focal points and eventually the entire bract may be enveloped.

Cellular degeneration in the peduncle follows the same general pattern as described for the receptacle, with the 2 points of origin being the epidermal region and the vascular tissue. Progressive stages of degeneration can be followed from the epidermis into the cortex, with the entire cortical area eventually being affected. The nature of the break-down of the epidermal and cortical cells is the same as described for the parenchymatous cells of the receptacle, i.e., agglutination of cytoplasm and degeneration of nuclei.

Response of the various cell types of the vascular bundles to the virus is characteristic. In the phloem of mosaic-diseased plants, the nuclei of the parenchyma and companion cells stain a deeper red than the healthy, are more particulate than their healthy counterparts, and ultimately degenerate in the characteristic fashion described previously. The cytoplasmic contents of these 2 cell groups, as well as the contents of the sieve cells, take a deeper stain, become

particulate, and eventually agglutinate into an amorphous mass. In conjunction with this nuclear and cytoplasmic reaction, the cells as a whole appear highly disorganized in outline (Fig. 2, D, E). The extent of phloem degeneration can be seen in Fig. 2, C. Brandenburg (1), working with another lettuce variety, reported the presence of wavy bodies in the sieve cells of mosaic-diseased plants; however, these structures were not seen in the material studied. The response of the medullary phloem to the virus is similar to that described for the phloem tissue (Fig. 2, E).

One of the most characteristic features of affected tissue is the production of a gummous material. The intercellular spaces of the pith, cortex, and developing bracts are frequently filled with this material. Also, the vessels and laticiferous elements of the receptacle are commonly plugged with the substance (Fig. 2, B). In many cases, when a floral head of a diseased plant is excised the latex exudate is a dark brown color rather than the characteristic white.

Degeneration of the parenchymatous cells of the medullary rays and pith appears to advance in a centripetal direction from the vascular bundles. Occasionally, necrotic areas that appeared to be isolated in their inception were found in the pith. However, these lay in close proximity to the medullary phloem and it is probable that these phloem strands served as the focal points of the degeneration.

Discussion.—The results show that individual plants of the variety Bibb vary in their seed transmission of lettuce mosaic virus. The percentage transmission listed for this variety, then, is actually an average figure for the variety as a whole, and does not necessarily indicate the degree of seed transmission for any given plant within it.

Whether or not the variation in virus seed transmission exhibited among the individual plants of this variety is due to differences in the genetic constitution of these plants, a chance distribution factor, or a combination of these 2, was not completely established by this work. However, the extreme differences in seed transmission of the virus found among individual plants of this variety, as well as the consistency in rate of seed transmission in line C through 3 generations, adds much to support the theory that the major contributing factor is genetic. In view of this, it is suggested that it might be possible to establish strains of lettuce that do not transmit lettuce mosaic virus through seed by the process of individual plant selection within already established varieties.

The random location of the viruliferous seeds throughout the inflorescences of the 2 plants checked indicates that, in the variety Bibb at least, the distribution of the virus to the floral heads is randomized, and that the activity of the virus remains fairly consistent throughout the growth of the plants, at least as far as infection of seeds is concerned.

The variety Cheshunt Early Giant has not been shown to transmit the virus through seed. It is possible that this phenomenon may be due to the hypersensitivity of floral tissue to a high level of virus activity. When the infectious level of the virus is presumably at its peak, the procambial tissue of the floral heads is obliterated, the epidermis degenerates, the vessels and laticiferous elements of the peduncle are plugged with a gummous exudate, and eventually the entire floral shoot is blighted.

With this overall blighting, there is an apparent decrease in virus activity, and a new group of floral shoots are initiated. That these shoots are infected is evidenced by the occurrence of the characteristic necrotic areas on the peduncles and bracts, and the production of local lesions on *G. globosa* inoculated with extracted juice from this tissue. However, this level of activity is half that of younger infected plants, and is not sufficient to induce complete breakdown of the later formed floral heads. Apparently the virus does not reach the developing embryo, or if it does, it evidently is not of sufficient infectivity to initiate the seed-borne condition.

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VARIETAL SUSCEPTIBILITY OF ALFALFA TO TWO SPECIES OF ROOT-KNOT NEMATODES [1]

Harold W. Reynolds [2]

SUMMARY

Ten varieties of alfalfa consisting of both northern (hardy) and southern (non-hardy) selections were rated in replicated tests, in the greenhouse, for their reaction to 2 root-knot nematode species, *M. javanica* and *M. incognita acrita*, commonly found in the Southwest. A second experiment was conducted in field plots, where a natural population of only one species, *M. incognita acrita*, was involved.

The greenhouse experiment showed that *M. javanica* was more damaging to most of the varieties than was *M. incognita acrita*. The 2 African selections were very resistant to either root-knot species. With the exception of the variety Narragansett, the northern varieties were more susceptible to either root-knot species than the southern varieties.

The 3 Chilean selections were intermediately susceptible to *M. javanica* but quite resistant to *M. incognita acrita*.

Alfalfa fields in most sandy-textured soils in the Southwest are known to be infested with root-knot nematodes, *Meloidogyne* spp. Göldi. The degree of infection has varied from very light to moderately heavy depending upon several factors, namely soil texture, cropping practice prior to seeding alfalfa, number of years that the field in question has been farmed, and variety of alfalfa grown. The nematodes attack the fine feeder roots and cause a marked decrease in length and number of roots, and hence in the foraging ability of the plant. The pest does not always cause conspicuous galling on alfalfa roots as it generally does with other hosts. Therefore, a casual field examination may not necessarily reveal the presence of the pest. In certain instances, however, where a high root-knot population is involved at the time of seeding the crop, and when several larvae enter the root at one point, galls may be formed on both primary and secondary roots.

Willhite and Smith (5) observed that heavy root-knot infections influence the type of root systems and the quantity and quality of forage produced. They further demonstrated a significant increase in yield of hay from field plots in which the pest had been controlled through soil fumigation. Thorne (4) observed that in root-knot infested fields, alfalfa stands failed to become well established, the root systems were branched and spreading, and few long tap roots were developed. He further noted that in plots plowed up after 4 or 5 years in alfalfa, less than 10 per cent of the roots were growing deep enough to be cut off by the plow. The young seedlings had been attacked early in life and tap root production practically eliminated. As the tap roots were destroyed, laterals were produced and these in turn were attacked and still further stunting and multiplication of roots followed. The writer observed a field of Ranger alfalfa near Yuma in which many seedlings had died 3–4 weeks after seeding. This field, however, had been seeded to alfalfa immediately following a crop of sesbania that had been infected with the Javanese root-knot nematode, *M. javanica* (Treub) Chitwood, the predominant species in this area. This field as well as others in which Ranger had been seeded were again examined after 1 and 2 seasons of growth, and heavy infections were found. At Sacaton, Arizona, where the cotton root-knot nematode, *M. incognita acrita* Chitwood, is the predominant species, very light infections were found in plots and fields in which Chilean alfalfa was growing. Consequently, these differences in the amount of infection on certain varieties of alfalfa where different species of root-knot are involved led to further

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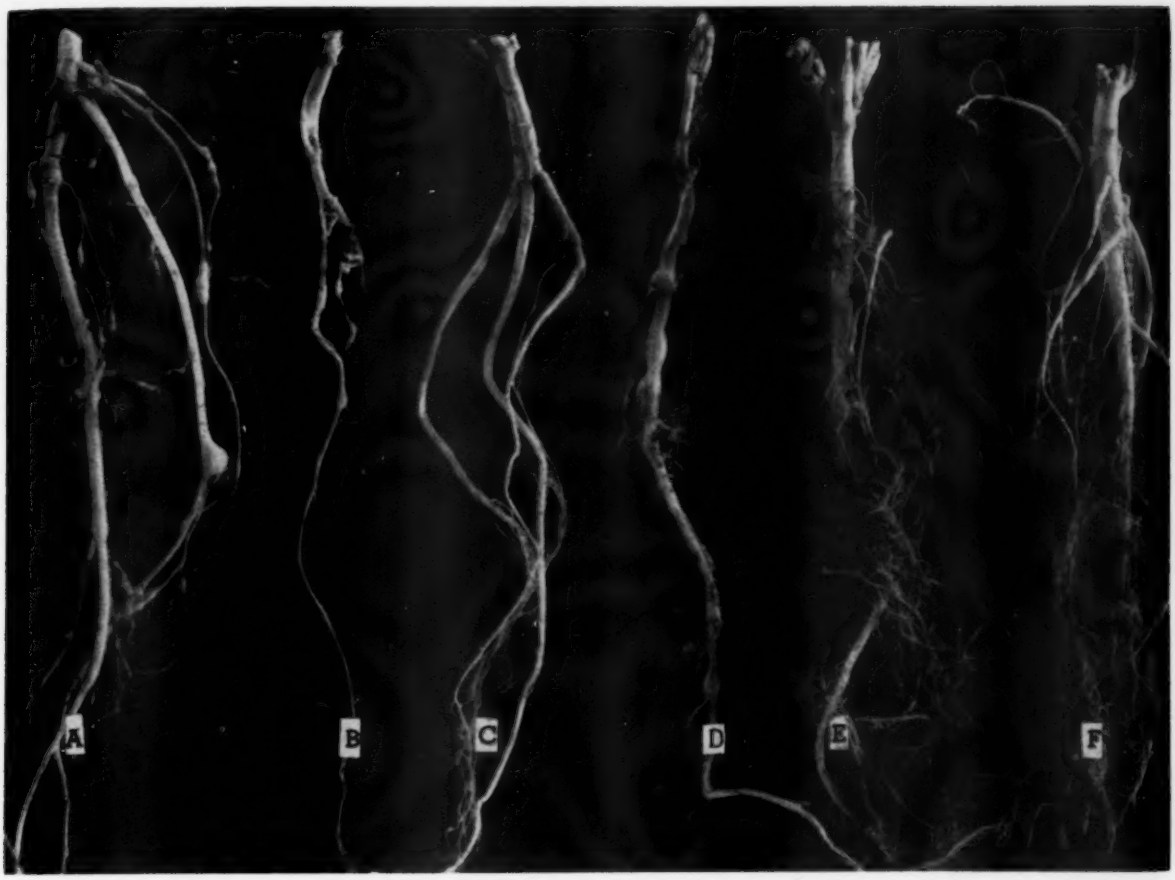

FIG. 1. Alfalfa roots showing variation in susceptibility and resistance to 2 species of root-knot nematodes. A) Nemastan variety with galling and absence of feeder roots resulting from attack of *M. javanica*. B) Ranger variety damaged by *M. javanica*, root system poorly developed and galled. C) African variety not damaged by *M. javanica*, good root system. D) Chilean (common) variety with root galls and few feeder roots after attack by *M. javanica*. E) African variety with no galling from *M. incognita acrita*, numerous feeder roots. F) Chilean (common) variety showing very few galls from *M. inocgnita acrita*, numerous feeder roots.

studies on susceptibility of alfalfa varieties to the 2 root-knot species concerned.

MATERIALS AND METHODS.—*Greenhouse experiment.*—Eighty pots containing pasteurized soil were embedded in sand in the greenhouse bench. To provide relatively uniform heat and almost optimum temperatures for development of the root-knot nematode, relatively high soil temperatures of 28°C during the daytime to 24°C at night were maintained by means of an electric heating cable embedded in the sand beneath the pots. Forty pots were inoculated with the Javanese root-knot and 40 with the cotton root-knot nematode. Large populations of each of the 2 root-knot species had been developed from single egg masses by using tomato as the host for the Javanese root-knot and cotton for the cotton root-knot species. The inoculum, which consisted of viable larvae in water, was secured in accordance with the procedure outlined by Godfrey (1) and later modified by Reynolds (2). Approximately 1400 larvae were added to the soil of each pot just before the alfalfa was seeded. Each group of 40 pots was spaced sufficiently far from the other group to prevent contamination through watering or larval migration.

Each group of 40 pots was seeded with 10 varieties of alfalfa and randomized in 4 blocks. Approximately 20 plants were grown in each pot for a period of 4 months. At that time all pots were taken to the laboratory and the soil was washed from the roots by means of a fan nozzle, using low water pressure. A relative root-knot index as described by Smith and Taylor (3) was determined for each plant with the aid of the dissecting microscope.

Field plot experiment.—A portion of a field at the U. S. Field Station, Sacaton, Arizona, where only the cotton root-knot nematode has been found was selected for this experiment. The soil is a coarse textured sandy loam very favorable for root-knot development. A moderately high root-knot population was built up within a period of 4–5 months by growing sun hemp, *Sesbania macrocarpa*, on the land. Eight varieties of alfalfa were seeded in randomized blocks with 4 rep-

TABLE 1.—*Relative susceptibility of 10 varieties of alfalfa to 2 species of root-knot nematodes (Greenhouse pot experiment—controlled conditions)*

Variety	Relative root-knot index [a] Javanese root-knot (Average of 4 replications)	Cotton root-knot (Average of 4 replications)
African	0.30	0.38
African (Bryan's Selection)	0.31	0.42
Narragansett	1.38	1.19
Chilean 21-5-5	2.44	0.70
Chilean 21-5	2.80	0.85
Chilean (common)	3.06	0.85
Buffalo	3.36	2.25
Atlantic	3.39	1.91
Nemastan	3.48	2.06
Ranger	3.55	2.32
L.S.D., 5%	0.25	0.28
L.S.D., 1%	0.34	0.37

[a] Relative root-knot index based on:
0 = No infection
1 = Trace of infection
2 = Light infection
3 = Moderate infection
4 = Heavy infection

TABLE 2.—*Relative susceptibility of 8 varieties of alfalfa to the cotton root-knot nematode after 2 years of growth (Field plot test—natural root-knot population)*

Variety	Relative root-knot index (Average of 4 replications)
African	0.23
Chilean (common)	0.53
Chilean 21-5-5	0.60
Hairy Peruvian	1.03
Atlantic	1.80
Buffalo	2.18
Ranger	2.68
Nemastan	2.95
L.S.D., 5%	0.49
L.S.D., 1%	0.66

lications. Each plot was approximately 100 sq. ft. in size. After the plants had grown about 2 years, 10 plants were carefully removed from each of the 32 plots and brought to the laboratory for further study. Any adhering dirt was washed from the roots and a relative root-knot index was made of each plant with the aid of the dissecting microscope.

EXPERIMENTAL RESULTS.—When the same varieties of alfalfa were inoculated with equal numbers of larvae of 2 root-knot species, the Javanese root-knot caused more damage than the cotton root-knot nematode to all varieties except African and Narragansett (Table 1 and Fig. 1). Both selections of the variety African were very resistant to either species of root-knot in greenhouse pot studies. The one selection of African was also resistant under field plot conditions where a natural population of the cotton root-knot nematode was present (Table 2). With the exception of the variety Narragansett, the northern varieties were more susceptible to either root-knot species than the southern varieties, namely African and Chilean. The 3 Chilean selections were susceptible to the Javanese root-knot but quite resistant to the cotton root-knot nematode.

DISCUSSION.—Alfalfa is an important crop in the Southwest, not only as a cash crop but also as a soil builder. In order to maintain soil fertility at a high enough level for good cotton yields, growers rotate cotton with alfalfa. In addition to increased cotton yields following alfalfa, a marked decrease in root-knot damage to cotton has been reported especially during the first and often the second year. This is attributed in part to the fact that the Chilean and African varieties are quite resistant to the cotton root-knot nematode and therefore only a very light infestation will be present as an initial population at the time the cotton is seeded. Furthermore, the characteristic deep rooting of the alfalfa plant is an aid to spreading out, rather than concentrating, a root-knot nematode population in a soil. In other words, root-knot larvae distributed in soil down to approximately 5 ft. do not as readily encounter the roots of the succeeding more shallow rooted cotton plant.

It is fortunate that the Javanese root-knot nematode, which readily attacks all alfalfa varieties tested except African, does not damage cotton. Almost the entire population of this nematode species may disappear during the years in which cotton is grown, provided weed hosts are controlled in the cotton fields. If the Javanese root-knot species were a problem in cotton production, many more fields heavily infested with root-knot would result because a large acreage of Chilean alfalfa is grown in rotation with cotton.

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PHYSIOLOGIC RACES IN USTILAGO NUDA AND TECHNIQUES FOR THEIR STUDY[1]

V. F. Tapke[2]

SUMMARY

Four physiologic races of *Ustilago nuda* were isolated on the basis of their reactions on the differential varieties Charlottetown (C. I. 2732), Compana (C. I. 5438), Freja (C. I. 7130), Stephan (C. I. 8051), and Valki (C. I. 5748). A total of 143 nuda collections from 30 states, 2 from Canada, and 1 from Mexico were identified as to race. Race 1 comprised 63 per cent of the total collections and was widely distributed. Race 4 was common in the southern states and may be important on winter barleys.

Six 2-row spring barleys tested 2, 3, or 4 years, were immune from all races: Abyssinian (C. I. 668), Bifarb (C. I. 3951-3), C. I. 3694, C. I. 5798, Jet (C. I. 967), and Kitchin (C. I. 1296). Jet also was immune from all of the writer's 9 races of *Ustilago nigra* and from 7 of *U. hordei*. Against the remaining 6 races of the latter, Jet developed from 2–12 per cent smutted heads.

The resistance or susceptibility to *U. nuda* of 148 spring and 103 winter barleys is listed.

The loose smut fungus *Ustilago nuda* infects barley only through the flowers. Artificial inoculation of barley flowers with *U. nuda* often results in wide variation in infection. In the present study of physiologic races of the pathogen, techniques applied to stabilize infection of the differential varieties were as follows: 1) Most of the floral inoculations were made in a greenhouse under controlled conditions to avoid the influence of variable outdoor climate on infection during the bloom period of 2 weeks or more. 2) Only 2-row barleys were used as differential varieties to reduce or avoid variability in infection due to wide differences in the stage of development of flowers within a head of 6-row barley. 3) Only uniformly fresh, highly viable chlamydospores obtained from greenhouse plants were used as inoculum.

The normally short-lived chlamydospore collections were partly viable after storage for 12–13 years at 28–32°F. Up to 90 per cent of smutted heads were produced by seeds from flowers inoculated with spores that had been kept for 7 years at 28–32°F. Up to 73 per cent smutted heads was produced by plants from seed stored 7 years at 28–32°F. after artificial inoculation of the flowers.

A maximum of 9.4 per cent infected heads was obtained through use of a new method of seed inoculation with *Ustilago nuda* chlamydospores.

Thirteen 2-row spring barleys were florally inoculated with each of 19 collections of *Ustilago nuda* to determine injury from loose smut. At the extremes, Goldfoil (C. I. 928) produced approximately 66 established plants from each 100 inoculated flowers, whereas Kutan (C. I. 1466) produced only 21.

The deep-infecting loose smut [*Ustilago nuda* (Jens.) Rostr.] takes an appreciable toll of barley in the United States year after year. Little is done to combat it because it rarely causes spectacular losses, the smutted heads disappear soon after emergence, causing the damage to be underestimated, and the disease is unusually difficult to control. The causal fungus infects barley only through the flowers, then grows deeply into and within the developing seed. No thoroughly practical, effective seed treatment or other control measure on the farm has been found. Control through isolation of physiologic races of *U. nuda* and breeding for resistance to the complement of races, therefore, seems the logical procedure. Race isolation in *U. nuda*, however, presents difficult technical problems. This paper reports the results of experiments dealing with some of these problems.

PHYSIOLOGIC RACES IN USTILAGO NUDA.—Nahmmacher (9), Thren (23), and Oort (10) each found 2 races of *Ustilago nuda*, one associated with spring barleys, the other with winter varieties. Mohajir et al (7) refer to a common race and a Trebi race of *U. nuda* in Wisconsin. Skoropad and Johnson (16) mention 2 races, one capable and the other incapable of attacking the Titan variety. In a recent abstract Tapke (20) mentioned 5 races in the United States. Shortly thereafter Cherewick (3, 4) reported 10 races in Canada and also listed the reaction of 10 differential varieties to each race.

Oort (10) reported that varieties for differentiating physiologic races of *Ustilago nuda* are hard to find, and the writer has had a similar experience. Only 3 types of usable differential action were found among 75 varieties tested. These reactions are displayed by Charlottetown (C. I. 2732), Compana (C. I. 5438), and Valki (C. I. 5748). Freja (C. I. 7130) has a reaction like Charlottetown but blooms earlier and Stephan (C. I. 8051) has a reaction like Compana but is later. Both of these have been used in the present study as a protection against rain or other conditions unfavorable to infection that might occur during the inoculation period of Charlottetown or Compana.

All the above differentials are immune to 1 or more of the "nuda" races. The Golden Melon (C. I. 3192) variety is extremely susceptible to all collections and races tested to date, and therefore also was inoculated

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TABLE 1.—*Reaction of differential varieties of barley to 4 physiologic races of Ustilago nuda*

Race	Charlottetown (C. I. 2732)	Compana (C. I. 5438)	Valki (C. I. 5748)	Golden Melon (C. I. 3192)
1	S	R	R	S
2	S[a]	S	S	S
3	S	S	R	S
4	R	R	R	S

[a] S = Above 10 per cent smutted heads.
R = 0–10 per cent smutted heads.

as a check on the favorableness of conditions for infection, on viability of inoculum, and for preserving collections as viable mycelium in the seed.

REACTION OF DIFFERENTIAL VARIETIES TO 4 PHYSIOLOGIC RACES OF USTILAGO NUDA.—A total of 143 smut collections from 30 states, 2 collections from Canada, and 1 from Mexico were tested for race identification and 4 distinct physiologic races were isolated. The reactions of the differential varieties to these races are presented in Table 1.

Occurrence and distribution of races.—The occurrence and distribution of physiologic races of *Ustilago nuda* in 30 states, Canada, and Mexico are in Table 2.

As in the case of physiologic races in *Ustilago hordei* (18) and *U. nigra* (19), 1 race was more widely distributed and occurred far more frequently than any of the others. Country-wide, race 1 comprised 63 per cent of the total collections and was found in many states. Approximately ¼ of the collections were race 4. Races 2 and 3 were collected less frequently but doubtless are destructive at times. On the regional basis, occurrence of the various races presents a different aspect as shown in Table 3.

Race 4 was found as frequently as race 1 in the North Atlantic, South Atlantic and South Central States. Winter barley is commonly grown in these regions. Race 4 doubtless is important on this crop. In the spring-barley states of the North Central and Far Western areas, races 1 and 3 are the important ones.

Until recently, Trebi (C. I. 936) barley repeatedly has proved highly resistant to the nuda loose smut (11, 12, 13, 14, 25) and was used freely as a parent in breeding for resistance. The writer inoculated flowers of Trebi with races 1, 3, and 4 in the summer of 1947 and the seed was sown in a greenhouse the following September. Races 1 and 4 were innocuous but race 3 produced 84 per cent smutted heads. Also in 1947 Shands (15) collected a new race of loose smut that infected 62 per cent of Trebi plants at Madison, Wisconsin. The writer's study of this collection (race 2) showed that it differs from race 3 by its ability to infect Valki. The existence of races 2 and 3 may explain the experience with Titan, a Trebi derivative reported by Skoropad and Johnson (16). They state: "Until 1946, barley breeders at the University of Alberta made extensive use of the almost immune variety, Titan, in their breeding program. In that year an unprecedented percentage of infection was observed in Titan, which has subsequently increased to

TABLE 2.—*Occurrence of physiologic races in 146 collections of Ustilago nuda from 30 states, Canada, and Mexico*

	Race 1	Race 2	Race 3	Race 4
Alabama				2
Arizona	1			
California	1			
Colorado	10			
Georgia				1
Illinois	3			1
Indiana				1
Iowa	4		1	
Kansas	2			3
Kentucky				1
Maryland	2		1	3
Michigan	6		2	
Minnesota	7		1	
Missouri	3		5	
Montana	2			
Nebraska			1	
New York	4			1
North Carolina	4			3
North Dakota	10		2	
Oklahoma				2
Oregon	1			
Pennsylvania				7
South Carolina	1			
South Dakota	6		1	
Tennessee	5			3
Texas	3			3
Utah	1			
Virginia	7			4
Wisconsin	7	1		
Wyoming	1		2	
Canada	1		1	
Mexico		1		
Total Collections	92	2	17	35
Percentage	63.0	1.4	11.6	24.0

TABLE 3.—*Occurrence of races of Ustilago nuda in different regions of the United States*

Region	Race 1	Race 2	Race 3	Race 4
North Atlantic	4	0	0	8
North Central	49	1	13	5
South Atlantic	14	0	1	11
South Central	8	0	0	10
Far Western	17	0	2	0

TABLE 4.—*Reaction of barley varieties to U. nuda Spring barleys immune from 4 races*

Variety	C. I. No.	2 or 6 rows	Years tested
Jet	967	2	4
Abyssinian	668	2	3
Bifarb	3951-3	2	3
Kitchin	1296	2	3
———	3694	2	2
———	5798	2	2
———	4376	2	1
———	4408	6	1
———	4488	6	1
———	6378	2	1

a point where the variety must be classed as susceptible."

Varietal Resistance.—Many varieties of spring barley and some winter barleys were tested for resistance to loose smut. The results of these tests are in Table 4. Varieties listed as resistant developed some smut but less than 10 per cent smutted heads. Those listed as susceptible developed 10 to 100 per cent smutted heads.

Spring barleys resistant to 4 races.—C. I. 4173, C. I. 4177.

Spring barleys immune or resistant, but not tested against all races.—Anoidium C. I. 7269, Composite Cross Selection (Nebr. 381162) C. I. 7114, Ezond III-38-1, Ezond III-38-5, Ezond III-38-9, Ezond III-38-12, Ezond III-38-17, Flynn × Glabron (Nebr. 383962) C. I. 7262, Glabron × Ezond (Nebr. 383576) C. I. 7263, Ogalitsu C. I. 7152, Petaland × Vaughn (S. D. 317), Petaland × Vaughn (S. D. 391), Plains C. I. 7250, Wyoming 471 C. I. 8055.

Spring barleys susceptible to 1 or more races.—Alpha C. I. 959, Anatolian Black C. I. 2970, Arivat C. I. 6573, Atlas C. I. 4118, Atlas 46 C. I. 7323, Atlas × Trebi C. I. 5368, Atlas × Vaughn (M8) C. I. 6971, Atlas × Vaughn (M44) C. I. 6979, B 120-247-8 C. I. 7247, Balder C. I. 7131, Barbless C. I. 5105, Beecher C. I. 6566, Blackhull C. I. 878, Bonneville C. I. 7248, Chalet C. I. 1110, Club Mariout C. I. 261, Duplex C. I. 2433, Feebar C. I. 7260, Flynn 37 C. I. 5918, Flynn 1 C. I. 5911, Flynn Sel. (Ks. H. C. 41-112) C. I. 7259, Flynn × Glabron (Nebr. 383999) C. I. 7261, Flynn × Vaughn (Ks. H. C. 41-94) C. I. 7258, Frontier C. I. 7155, Gem C. I. 7243, Glacier C. I. 6976, Golden Pheasant C. I. 2488, Goldfoil C. I. 928, Good Delta × Everest (36 Ab. 5652) C. I. 7147, Hannchen C. I. 4841, Hannchen × Minia (36 Ab. 5117) C. I. 7156, Harlan C. I. 7008, Horn C. I. 926, Iowa H2-4-12-1 C. I. 7327, Iowa H12-3-11-14 C. I. 7326, Irisaka C. I. 1083, Kindred C. I. 6969, Kolter C. I. 987, Kutan C. I. 1466, Lico C. I. 6279, Lico 1 C. I. 7544, Lion C. I. 923, Lion × Minia C. I. 6980, Manchuria C. I. 2330, Manchuria C. I. 2947, Mars C. I. 7015, Michigan 2 row C. I. 2782, Minia × Golden Pheasant (36Ab2568) C. I. 7153, Minnesota II-37-7 C. I. 7257, Minnesota II-37-54 C. I. 7256, Montcalm C. I. 7149, Moore C. I. 7251, Moravian C. I. 7559, Morsett C. I. 4800, Munsing C. I. 6009, Newbly C. I. 2437, Nigrate C. I. 2444, O. A. C. 21 C. I. 1470, Odessa C. I. 934, Persicum C. I. 2249, Plains C. I. 7250, Plumage C. I. 2511, Plush C. I. 6093, Queens C. I. 7021, Rex C. I. 6618, South Dakota A C. I. 7253, South Dakota B C. I. 7254, Spartan C. I. 5027, Steigum C. I. 907, Trebi C. I. 936, Trebi × Dryland 69 C. I. 6981, Tregal C. I. 6359, Valentine C. I. 7242, Vance C. I. 4585, Vantage C. I. 7324, Velvon C. I. 6109, Velvon II C. I. 7088, Viner 1163 C. I. 6533, Weider C. I. 1021, White Smyrna C. I. 910, Wisconsin H12B52 C. I. 7144, Wisconsin X212-1 C. I. 7249 and the following unnamed varieties: C. I. 2258, C. I. 3212-2, C. I. 3345, C. I. 3668, C. I. 3946-1, C. I. 3957, C. I. 4517, C. I. 4559, C. I. 4729, C. I. 4742, C. I. 4756, C. I. 4879, C. I. 5797, C. I. 5800, C. I. 5856, C. I. 5866, C. I. 6029, C. I. 6382, C. I. 6390, C. I. 6704, C. I. 7153, C. I. 7154, C. I. 7156, C. I. 7244, C. I. 7245, C. I. 7246, C. I. 7322.

Winter barleys resistant but not tested against all 4 races.—C. I. 3210-5, C. I. 4966, C. I. 5324, Clancy C. I. 1002, Hooded 16 C. I. 6574, Kenbar C. I. 7574, Kinroku Sel. B467, Kinroku Sel. B468, M. E. B. Sel. B351, M. E. B. Sel. B404, M. E. B. Sel. B405, M. E. B. Sel. B411, M. E. B. Sel. B472, M. E. B. Sel. B502, M. E. B. Sel. B507, M. E. B. Sel. B538, M. E. B. Sel. B539, N. C. 26 C. I. 7026.

Winter barleys susceptible to 1 or more races.—Abundio C. I. 1412, Apsheron C. I. 5557, Arlsun-Wong-Jet, Black Russian C. I. 705, Black Russian C. I. 2202, Bougie C. I. 2479, Caucasus C. I. 4334, CCX-8 C. I. 7573, CCX-68-5 C. I. 8074, Colonial C. I. 8062, Denari C. I. 5225, Derbent C. I. 5008, Dicktoo C. I. 5529, Dobaku C. I. 5238, Dohadak C. I. 5187, Donjon C. I. 2266, Early Black Turkestan C. I. 3093, Ghest C. I. 979, Greece C. I. 221, Han River C. I. 206, Hokudo C. I. 5176, Iredell C. I. 6571, Kentucky No. 1, C. I. 6050, Khayyam C. I. 1117, Kido C. I. 5145, Kinroku Sel. B659, Kura C. I. 4306, Marm C. I. 5562, Meimi C. I. 5136, Omar C. I. 898, Peking C. I. 4202-2, Piedmont C. I. 8073, Purdue 28154 C. I. 7119, Randolph C. I. 6372, Reno C. I. 6561, Sabbaton C. I. 1266, Shonan C. I. 5255, Suchow C. I. 5091, Tenarab C. I. 662, Tennessee Winter C. I. 6034, Tucker C. I. 7039, Wong C. I. 6728 and the following unnamed varieties: C. I. 2349, C. I. 3356, C. I. 3358, C. I. 3367-1, C. I. 4290, C. I. 4298, C. I. 4299-1, C. I. 4299-2, C. I. 4301, C. I. 4302-1, C. I. 4308-2, C. I. 4310, C. I. 4313, C. I. 4314, C. I. 4316, C. I. 4326-1, C. I. 4326-2, C. I. 4327, C. I. 4329, C. I. 4348, C. I. 4757, C. I. 4952, C. I. 4953, C. I. 4959, C. I. 4972, C. I. 4973, C. I. 5006, C. I. 5007, C. I. 5018, C. I. 5559, C. I. 5564, C. I. 5565, C. I. 6389.

Jet (C. I. 967) has a remarkable record among the spring barleys listed above as immune. It has been immune from *Ustilago nuda* also in extensive tests by Shands and Schaller (14), Schaller (13), Mohajir et al (7), Skoropad and Johnson (16), and Konzak (5). In greenhouse tests against 9 races of *Ustilago nigra* (19) and 13 of *U. hordei* (18), in which Odessa produced up to 98 per cent smutted heads, Jet was immune from all races of *U. nigra* and from 7 of *U. hordei*. The remaining 6 races of *U. hordei*, produced only 2–12 per cent smutted heads in Jet. Ogalitsu, (C. I. 7152) also was outstanding in this test. One race of *U. nigra* caused 3.3 per cent smutted heads and one of *U. hordei* caused 3 per cent smutted heads but the variety was immune from all other races of both smut species. In the present study Ogalitsu was immune from races 1, 3, and 4 of *U. nuda* but has not been tested against the recently acquired race 2.

PROBLEMS OF TECHNIQUE.—*Varietal variability in smut reaction.*—Reasonably uniform reactions of the differential host varieties to the various races of smut are requisite to a study of nuda races. Several investigators have found that the susceptibility of varieties often varies considerably. Middleton and Chapman (6), who inoculated varieties of winter barley by the partial vacuum method of Moore (8), found that the

percentage of infected heads in Tennessee Hooded and N. C. Bearded varied from 3–72 and from 5–61.5, respectively. Ross et al (12) noted that the relative susceptibility of some varieties differed considerably with the season, and that the effectiveness of different methods of inoculation was influenced by the environmental conditions at flowering time. In periods of high humidity, floral inoculation was more effective with dry spores than with spores in aqueous suspension. Low humidity on the other hand favored the wet method. Temperature also influenced infection.

There has long existed a paucity of information concerning environmental and other factors that influence infection of barley and wheat by the loose smut fungi *Ustilago nuda* and *U. tritici*. One factor that may be involved in the variability of host reaction is the relatively wide spread in blooming time within a singel head of 6-row barley. When inoculating an entire head some of the flowers are not sufficiently advanced or are too advanced to be as susceptible as the flowers in the ideal stage of development. Taylor and Harlan (21) found differences between the lateral and central flowers of 6-row varieties in the degree of interlocking of glumes and consequently in the degree of susceptibility to *Ustilago nuda*. Only 2-row barleys were used as differential varieties in the present study. They are much easier to inoculate by the method used and there is much less spread in the stages of bloom among flowers in a head than in the 6-row sorts.

In the field, variability in host reaction doubtless also may be brought about by weather conditions that are favorable for infection during part of the blooming period and unfavorable at other times. Thus infection in plants from inoculated heads of early, medium, and late blooming differential barleys may reflect the vagaries of the weather instead of the true differential action of the variety. Therefore, in the present study, much of the work was performed under controlled greenhouse conditions at Beltsville, Maryland. The seed was sown in pots in January and flowers were inoculated in March and April. A high humidity and a temperature range of 65–75°F. were maintained throughout the period of inoculation. The field inoculations were made in June at Ithaca, N. Y. In September, seed from flowers inoculated in field and greenhouse were germinated at 68–72° in soil in small pans. Seedlings were transplanted to greenhouse benches a day or two after emergence. The plants received artificial light from midnight until 2 a.m. daily from late October until heading occurred in November and December when the smut records were taken. All emerging smutted heads were removed daily before the smut-enclosing membrances began to disintegrate or they were covered with glassine bags for possible future use. This system coupled with the use of 2-row differential varieties produced satisfactory results.

Floral Inoculation.—Shands and Schaller (14) summarized the results of experiments on the effectiveness of various methods of floral inoculation and the stage of flowering optimum for inoculation and infection. The ideal stage seems to be the day of pollination and 1 day before and after. All floral inoculations were made at this stage, in the present study. In the inoculations of 1950 and before, spores in aqueous suspension (½ gm spores in 1000 ml. water were inserted within each individual flower with a hypodermic syringe. Dry spores were used thereafter. One or more smutted heads of the race or collection under test were inserted in a small shell vial and cut into fragments. Sharp pointed forceps then were used to pick up a small quantity of the spore dust, pierce the glume and deposit inoculum on the stigma of each individual flower. Up to 100 per cent smutted heads and plants were obtained by both methods. The wet method requires too much time and the spores soon deteriorate in water, making daily preparations of fresh inoculum necessary. The dry spores maintain high viability throughout the period of inoculation if stored at 28–32°F. A change of dry inoculum involves only disinfection of the inoculating forceps in alcohol. Also a very small quantity of dry inoculum can be put on the stigma so that the common and often serious difficulty from shriveling and low germination of seed after overdosing the flowers with spores (12, 22, 25, 26) is largely avoided. The germination of 15,000 seeds from flowers inoculated with dry spores by the writer in 1952 averaged 71.3 per cent. Shands and Schaller (14) Schaller (13) and Mohajir et al (7) also found dry spores to be satisfactory for the artificial inoculation of barley flowers.

Collection and Storage of Inoculum.—The writer has received numerous field collections of barley loose smut from cereal workers throughout the country. The collections germinated from 0 to 100 per cent, upon receipt, and consisted of *Ustilago nuda* or *U. nigra* or a mixture of both species. It often was necessary to hold collections over until the following season despite the fact that chlamydospores of *U. nuda* normally retain their viability for only a few months at room temperatures (1). The spores also are prone to germinate more slowly as their viability declines. Since the barley embryo becomes immune from infection a few days after the stage for floral inoculations, the use of slow-germinating spores would be hazardous.

All smut collections were stored at a temperature of 28–32°F., which often prolongs their viability for many years. For example, chlamydospores of *U. nuda* received from Fort Collins, Colo., in 1938, were tested for viability in May 1951. Twelve per cent of the spores were germinating after 16 hours on 2 per cent potato-dextrose agar at 70–72°F. A 12-year-old collection from winter barley in Virginia, tested in May 1951, germinated 14 per cent. Numerous other collections of *U. nuda*, stored in glassine bags or paper envelopes, in a refrigerator, have remained viable for from 1–10 years.

The stored collections also were able to produce infection. Fifteen stored collections received in 1944 were used to inoculate flowers of the highly susceptible Atlas (C. I. 4118) spring barley in April, 1951. The

resulting seed was sown in a greenhouse the following September and produced from 10 to 90 per cent of smutted heads.

In another experiment, seed of 4 spring barleys from flowers inoculated in 1940 was sown in a greenhouse after storage at 28–32°F. for 7 years. As shown in Table 5, satisfactory percentages of seed germination and smut infection were obtained. The 2 varieties having the highest seed germination had the lowest infection of smut. Previously, Brefeld and Falck (2) and Zimmerman (27) had found *U. nuda* mycelium to be viable in seed 2 and 3 years old.

Collections of spores or of mycelium in the seed from inoculated flowers were stored at 28–32° except when actually in use. New Collections first were germinated on 2 per cent potato-dextrose agar to identify the species. The nuda species in mixtures of *U. nuda* and *U. nigra* was isolated by a process previously described (17). As soon as possible the new collections were used to inoculate a highly susceptible spring variety such as Atlas (C. I. 4118) or Odessa (C. I. 934) under controlled greenhouse conditions favorable for infection. The smut collection then was maintained as mycelium in the seed. When inoculum was needed the infected seed was sown in a greenhouse early in January, and fresh, highly viable spores from the infected plants were available for inoculating the flowers of greenhouse and field plants in April and June, respectively.

Injurious effects of floral inoculation.—The artificial inoculation of barley flowers frequently has been reported to be harmful to the set and germination of seed, and some varieties seem to suffer more than others (14, 22, 25). The writer florally inoculated 13 spring 2-rowed varieties with each of 19 nuda collections at Ithaca, N. Y., in June 1946 by injecting a few drops of aqueous spore suspension into each flower. In September the inoculated heads were thrashed by hand and 11,000 seeds were sown in soil in small pans and germinated at 20°C. The seedlings were transplated to green-house benches upon emergence. The seed set and seed germination, and the survival of seedlings 1 month after transplanting were recorded for each of the 247 lots. The seed set, seed germination and seedling survival of Goldfoil (C. I. 928) were 84, 89, and 89 per cent, respectively, while the respective percentages for Golden Pheasant (C. I. 2488) were 60, 72, and 68. Both varieties were highly susceptible to all 13 nuda collections. The inoculated heads of Goldfoil averaged 16.6 established seedlings while Golden Pheasant and Kutan (C. I. 1466) averaged 7.3 and 5.2, respectively. The other varieties fell between these extremes. Varieties that suffer too much injury are unsatisfactory as differential hosts.

TABLE 5.—*Germination and smut infection of barley from seed stored 7 years at 28–32°F. Seed from flowers hand-inoculated with Ustilago nuda*

Variety and C. I. Number	Number of seeds sown	Percentage of seed germination	Number of plant heads	Percentage of smutted heads
Lion (923)	203	46	139	73
Alpha (959)	80	59	58	64
O.A.C. 21 (1470)	213	78	277	18
Manchuria (2330)	87	87	91	17

FIG. 1. Atlas (C. I. 4118) barley (left) produces a more desirable type of smutted head than does the highly susceptible Odessa (C. I. 934) variety (right) for the increase of inoculum under greenhouse conditions.

Seed inoculation.—Physiologic races of *Ustilago nuda* could be studied more effectively if a practical method could be devised for obtaining infection through inoculation of the seed. Apparently no one but Vanderwalle (24) has been successful previously. He injected a mycelial suspension of *U. nuda* into the barley embryo with the aid of a micromanipulator. In 1950 and 1951 the writer soaked smut-free seed of Compana (C. I. 5438) in water at room temperature for 24 hours, and then split the seed lengthwise by inserting a scalpel into the upper end of the crease. The 2 halves were gently pulled apart and the embryo usually adhered to 1 of the halves. The exposed embryo and endosperm then were dipped in an aqueous

TABLE 6.—*Loose smut in Compana (C. I. 5438) barley as a result of inoculating seed (from which half of the endosperm had been removed) with chlamydospores of Ustilago nuda*

Test year	Inoculated			Non-inoculated check		
	Seed	Seedlings	Percentage of Smutted plants	Seed	Seedlings	Percentage of Smutted plants
1950	100	96	9.4	100	100	0
1951	100	97	7.2	100	98	0

spore suspension (1 gm. of fresh, highly viable spores in 1000 cc. sterile water) of physiologic race 3. Following inoculation, the seed was sown ½ in. deep in sterile soil in a small pan and germinated at 72–74°F. The seedlings were transplanted in a greenhouse bench several weeks after emergence. The lot of Compana barley used had been grown from smut-free seed at Aberdeen, Idaho, where loose smut rarely occurs, and several previous tests of this lot gave only smut-free plants. Results of the experiment are in Table 6.

The above method of seed inoculation is not difficult and it is not appreciably injurious to germination when the seed is sown in sterile soil, but the percentage of smut infection is too low. Compana normally produces 80–100 per cent smutted plants when florally inoculated with this race.

Atlas barley for preserving and increasing smut collections.—In studies of physiologic races of *Ustilago hordei* and *U. nigra* previously reported (18, 19), the writer found Odessa (C. I. 934) spring barley to be highly susceptible to all collections and races of both species and therefore useful for producing a supply of inoculum. Odessa, likewise, has been highly susceptible to all collections and races of *U. nuda* tested to date. As shown in Fig. 1, however, the plants often become so severely infected that good smut heads are not obtained under greenhouse conditions. The variety Atlas (C. I. 4118) produces better smutted heads and at least to date has been highly susceptible to all "nuda" collections and races with which it has been florally inoculated.

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PRODUCTION AND ROLE OF EXTRACELLULAR PECTIC ENZYMES OF FUSARIUM OXYSPORUM F. LYCOPERSICI [1]

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SUMMARY

Pectin methylesterase (PM) is and polygalacturonase (PG) is not excreted by *Fusarium oxysporum* f. *lycopersici* growing on glucose media. Both enzymes are produced by the fungus growing on a pectin medium. Maximum enzyme concentrations and mat weights are attained *in vitro* at about the same time. PG disappears from the medium before PM.

The PG and PM of Fusarium can be adsorbed on Celite and eluted separately with a degree of purification. The PG has about the same characteristics as other fungal PG. However, PM is unusual in that it is relatively active at pH 7. The PG is more active on pectin in the presence of PM. However, PM in the absence of PG does not reduce the viscosity of pectin or macerate tomato stem tissue. Galacturonic acid is produced by the PG. PM from Fusarium and probably PG are present in the vascular stream of diseased tomato plants and must be active during pathogenesis.

Preparations of the enzymes so far made may contain contaminants. Therefore, their action on tomato cuttings cannot be taken as a demonstration of the role of pectic enzymes in the production of tomato wilt.

Extracellular pectic enzymes produced by *Fusarium oxysporum* f. *lycopersici* may play an important role in the tomato wilt syndrome. In this paper are discussed the characteristics of these enzymes, their production rates *in vitro* and *in vivo*, and the effect of purified preparations upon the host.

Arguments can be advanced concerning the role of these enzymes. Wilting[2] and vascular discoloration are prominent symptoms in tomato wilt. Wilting has been attributed to the plugging material seen in invaded vessels (25). Any molecule, if sufficiently large, could lodge in vessels. Of the natural materials likely to be present, polysaccharides of high molecular weight (18) and the pectic compounds (19) are possibilities. In addition, pectic compounds can form calcium pectate gels. Subsequent studies on diseased plants have suggested that pectic compounds are present in vascular elements (25). Gums have been seen to appear in diseased plants at the expense of the pectin-laden middle lamella (17).

Pectic compounds in vessels could arise by pectic enzymes excreted by the pathogen acting on pectic compounds at pit membranes where the middle lamella is exposed (22). A partial hydrolysis of pectins to substances of intermediate molecular weight could result in calcium pectate gels, which would obstruct flow in vessels. Alternatively, gums might be formed from the synthetic action of enzymes upon the product of pectin hydrolysis (30).

Vascular discoloration is thought to arise by the oxidation of the polyphenols that are found in infected tomato vessels (5). When potato stems are injured in a localized area by steam, the vascular bundles above become discolored (16). Presumably polyphenols are released from the injured cells into tracheal elements. The materials leading to discoloration, which are liberated from steamed cells, might also be liberated through maceration of tissues by pectic enzymes. Phenolic glycosides, released from these cells, would be split by glycosidase, which is produced by both the fungus and the host, releasing the discoloring polyphenols (5).

Recently, culture filtrates and commercial enzymes high in pectin–methylesterase (PM) have been shown to cause wilting and vascular discoloration in tomato cuttings (14, 39). Although some progress in purification was made in our studies, contaminating substances undoubtedly still exist in the preparations to which we exposed tomato cuttings. Therefore, it must be borne in mind that mere demonstration that a culture filtrate contains components that lead to, say, wilting of cuttings is insufficient to show that these components are involved in diseased plants (3, 6, 15, 19, 34, 37).

METHODS.—*Cultural methods.*—Nutrient media contained 0.1 per cent NH_4NO_3, 0.1 per cent KH_2PO_4, 0.05 per cent $MgSO_4$, and 1.5 per cent carbon source. The carbon source was either A. R. glucose (glucose medium) or practical grade pectin[3]. In some studies, 1.5 per cent casein hydrolysate was substituted for NH_4NO_3 and 1 per cent yeast extract was added (glucose-casamino acids medium). Media were inoculated with a pathogenic strain, R5-6, of *F. oxysporum* f. *lycopersici* and cultures were grown in either shake or quiet culture at room temperature. At the end of the growth period, mycelium was collected on coarse filter paper and weighed after washing and drying at 37°C. The culture filtrate was retained for enzyme assays. Bran cultures were grown and extracted by the method of Winstead and Walker (39).

Preparations were dialyzed 4 hours against running

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[2] Wilting in this paper means simple loss of turgor.

[3] Pectin and pectic acid were supplied through the courtesy of California Fruit Exchange, Pectinol 100D and Pectin Esterase No. 5 by Rohm & Haas Co.

tap water and overnight against more than 10 volumes of distilled water.

Protein assay.—Protein was determined by measurement of the light absorption at wavelengths 280 and 260 mμ according to the method of Warburg and Christian (36). Proteins in turbid filtrates were estimated by summing the contents of clarified solutions eluted from Celite.

Pectin-methylesterase (PM) assay.—The PM assay depends upon the number of milliequivalents (m.e.) of ester groups hydrolyzed per minute per ml of enzyme solution. Ten ml of enzyme preparation was added to 10 ml of 1 per cent pectin (10.3 per cent methoxyl groups) and 1 ml of 2 N NaCl containing methyl red and the solution adjusted to pH 5.2. The assay was carried out at 37°C for 20 minutes with the enzyme concentrations adjusted to give 10 per cent or less hydrolysis. The number of m.e. of ester groups hydrolyzed was determined by titrating with 0.02 N alkali. Over this range the number of m.e./ml/minute was directly proportional to concentration of enzyme. Variability introduced by the assay and by growth of the fungus resulted in a standard error of 18 per cent for a mean of 2 cultures.

At other pH values, assays were conducted for 15 minutes at 25°. Then the solution was titrated in 2 minutes to pH 7. From the total quantity of alkali used was subtracted the quantity required to titrate and identical, nonincubated mixture from the given pH to 7. This gave the m.e./minute/ml hydrolyzed at the given pH (28).

Low PM concentrations were assayed by mixing 1 ml of the preparation with 2 ml of 0.05 M NaCl solution containing 0.25 per cent pectin and methyl red. The pH was adjusted to give about the same optical density at 520 mμ as 1 ml water plus 2 ml substrate and indicator solution at pH 5.0. For concentrations of Pectinol 100D less than 0.05 per cent, the change in optical density in 10 minutes was linearly related to concentration.

Polygalacturonase (PG) assay.—The cup plate assay for PG (8) was modified by substituting 0.2 M ammonium acetate buffer for phosphate buffer. The pH of the medium was 5.4, the optimum for alpha-PG (8, 33). A sample of Pectinol 100D was employed as a standard, 0.05 ml of a 1 per cent solution containing 1 unit and producing a zone of about 2.5 cm diameter.

Change in viscosity of solutions was determined at 30° in size 100 Fenske-Ostwald viscosimeters and expressed as a percentage of the total possible change from pectin to galacturonic acid solution. Reducing groups were estimated by the hypoiodite method (38).

Glucosidase assay.—One quarter ml of enzyme preparation was mixed with 2.5 ml 0.002 M salicin in 0.1 N acetate buffer (pH 5), 0.125 ml phenol reagent (10), and 0.5 ml 20 per cent Na_2CO_3. The solution was heated 1 minute, cooled immediately, and the optical density determined at 650 mμ. A second sample was incubated for 24 hours at 30° and its phenol content determined similarly. The difference in optical densities of the 2 provided an index of glucosidase activity.

Separation of enzymes.—Although 60 per cent saturation of 8-day quiet culture filtrates with ammonium sulfate precipitated about half of the glucosidase, not much PM or PG was precipitated. However, the addition of 3 volumes of acidulated ethanol after adding a pinch of pectin (21) precipitated high yields of PG, PM and glucosidase.

A high percentage of orange flavedo or alfalfa PM is adsorbed on Celite and eluted by M NaCl (24, 27). However 0.025 M Na_2HPO_4 elutes only a small percentage (27). Fusarium PM behaves similarly whereas PG does not, thus permitting a separation of the 2 pectic enzymes.

One hundred ml of raw filtrate or 10 ml of a 10-fold concentration of the enzymes obtained by alcohol precipitation (21), pH 5–6, was dialyzed and passed through 2 g of acid-washed Celite on filter paper in a Buchner funnel. Less than 10 per cent of PG or PM pass through (Table 1). Ten ml lots of 0.025 M Na_2HPO_4 are then passed 2 times through the Celite. High concentrations of PG accompanied by low concentrations of PM are eluted. After elution with 6 10-ml lots of phosphate, treatments with 10-ml quantities of M NaCl elute high concentrations of PM. High concentrations of glucosidase accompany the PG eluted with Na_2HPO_4 but not the PM eluted with NaCl. Although the raw filtrate is turbid and blue,

Table 1.—*Concentration and separation of enzymes by Celite method*

	Volume, ml	Percentage of initial activity/ml			Protein, mg/ml
		PM	PG	Glucosidase	
A. Ethanol precipitated Fusarium filtrate[a]					
Filtrate through Celite	10	2	1	21	0.12
1st Na_2HPO_4 eluate	10	1	26	53	0.19
6th Na_2HPO_4 eluate[b]	10	0	8	1	0.08
1st NaCl eluate	10	15	2	0	0.03
B. Raw Fusarium filtrate[c]					
Filtrate through Celite	78	2	1	....	0.16
1st Na_2HPO_4 eluate	20	4	205	90	0.24
3d Na_2HPO_4 eluate	20	4	46	....	0.09
1st NaCl eluate	30	46	5	10	0.04
C. 1 per cent Pectinol 100D[d]					
Filtrate through Celite	90	95	37		0.74
1st Na_2HPO_4 eluate	20	53	33		0.36
3d Na_2HPO_4 eluate	20	10	8		0.10
1st NaCl eluate	30	10	3		0.04

[a] Alcohol precipitated culture filtrate contained PM that hydrolyzed 98×10^{-5} m.e./ml/minute at 37°, pH 5.2; PG activity of 1.65 units by cup plate assay.

[b] Sixth elution with 10 ml 0.025 M Na_2HPO_4. Intermediate fractions showed intermediate activity.

[c] Raw filtrate contained PM to hydrolyze 54×10^{-5} m.e./ml/minute and PG activity of 0.18 units by cup plate assay.

[d] Pectinol 100D contained PM to hydrolyze 78×10^{-5} m.e./ml/minute and PG activity of 0.78 units.

the eluates are clear. The gain in PM activity per mg protein is more than 3- to 5-fold, in PG more than 1- to 3-fold.

PM and PG in a commercial preparation, dialyzed Pectinol 100D, were not adsorbed by Celite in the same manner as the Fusarium enzymes (Table 1). Other evidence of differences between PM from the 2 sources is presented below.

CHARACTERISTICS OF PECTIC ENZYMES IN VITRO.—*Effect of pH.*—The pH optimum of PG from Fusarium was compared with that in Pectinol 100D, using the cup plate assay. The pH of the agar-pectic acid medium was adjusted to different levels before plates were poured. The optimum for both the PG from Fusarium and in Pectinol 100D was pH 4.5–4.8 (Fig. 1). Similar results were obtained with PG from other shake and from quiet pectin cultures. Others (1, 8) obtained similar results for preparations containing both alpha-PG and beta-PG.

The effect of pH on the activity of PM from Fusarium was also determined (Fig. 2). The enzyme in raw filtrates of a 5-day shake pectin culture or after purification on Celite from a 10-day quiet culture is as active at pH 5 as at pH 7. This contrasts with the PM from Pectinol 100D which is much more active at pH 5 than at pH 7 (28). Thus the PM from the 2 sources differ in at least 2 ways: Fusarium PM is adsorbed by Celite and is relatively active at pH 7 while Pectinol PM is not adsorbed and is inactive at pH 7.

Temperature.—Stability of the enzymes is affected by other materials in solution. Nondialyzed filtrates from a 4 day shake pectin cultre and from a 21-day quiet pectin culture of Fusarium were heated to 50°C at pH 5.3 and the first order inactivation rates determined. PG was assayed by use of cup plates. At this temperature, the inactivation rate of PG from shake cultures was 3.5 times as fast and from quiet culture was 5.5 times as fast as for PM produced under the same conditions. Since no inhibitor was found in filtrate from older cultures, the disappearance of PG from cultures while PM remains (Fig. 4) is probably a result of inactivation of PG.

Stability in the presence of salts was also determined for the 2 enzymes in the nondialyzed fractions from the Celite separation. The PG in the first 20 ml phosphate eluate and the PM in the first 30 ml of NaCl eluate were each adjusted to pH 6. No difference in decomposition rate was detected at 35°, but PG decomposed 8.3 times as rapidly at 45° and more than 6 times as rapidly at 52° as PM.

The enzymes behave quite differently in dialyzed preparations. The losses in activity at pH 6 of PM from Pectinol 100D and Fusarium (NaCl elution from Celite) and of PG from Fusarium (phosphate elution from Celite) are shown in Table 2. PMs from the 2 sources are similar to each other and to the fungal PM examined by McColloch and Kertesz (28). Fusarium PG showed about the same loss of activity whether assayed by the cup plate method or with the viscosimeter. PG, which was relatively unstable in salt solutions, was more stable than PM in solutions of low salt concentration.

TABLE 2.—*Enzyme activity after heating dialyzed preparations at pH 6. Expressed as percentage of activity in unheated aliquot*

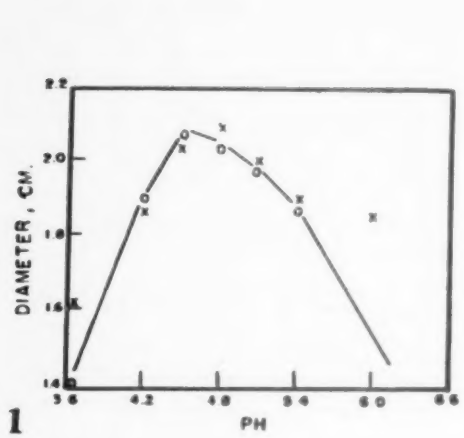
Temperature, °C.	35	45	55
Time, hours	4	1	1
PM: Pectinol	15	—	5
Fusarium	20	8	1
PG: Fusarium, cup plate	65	52	1
viscosity	76	58	1

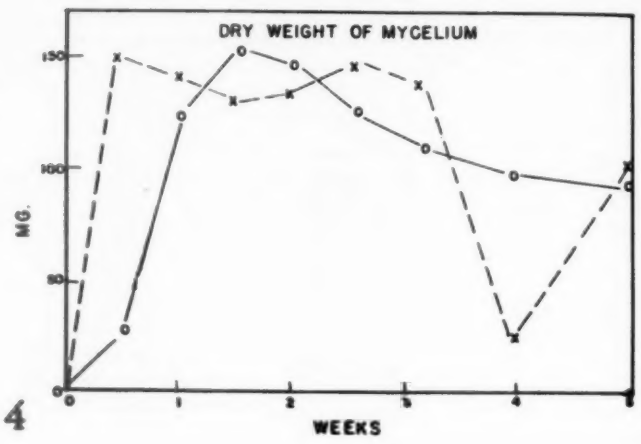
Inhibitors.—PM purified from Fusarium cultures was not inhibited at 37° and at pH 5.2 by the presence of 0.03 per cent Triton 720, a wetting agent. In this respect it does not differ from other fungal PM (28). In a similar assay, the activity of PM extracted from tomato stems was sharply reduced by Triton 720.

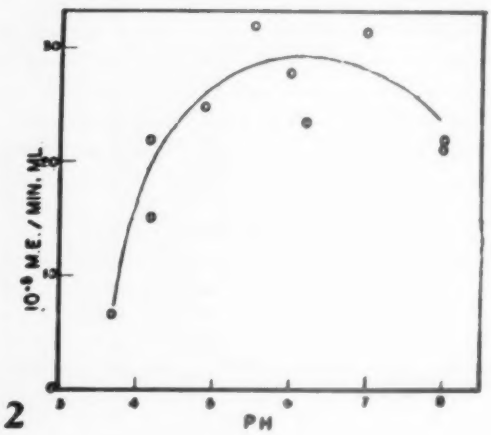
Effect of esterification of substrate upon PG activity.—Fusarium PG preparations behaved in the cup plate assay as alpha-PG, i.e., it produced a zone of hydrolysis on pectic acid agar at pH 5.3. Beta-PG requires the presence of PM to hydrolyze pectin (8, 20). To test for beta-PG, a solution containing 1.4 units of PG (cup plate assay) and 0.19 mg protein/ml was prepared by the Celite method from a culture filtrate. This preparation had a PM activity of only 4×10^{-5} m.e./ml/minute, 5 per cent of the activity of the initial filtrate. In a viscosimeter, 1 ml of this preparation with 1 ml of water and 10 ml of 0.33 per cent pectin in 0.2 M acetate buffer, pH 4.4, reduced the viscosity 30 per cent in 20 minutes. In contrast, the addition of 1 ml of 1 per cent Pectin Esterase No. 5 to 1 ml of Fusarium PG and 10 ml of substrate caused a drop in viscosity of about 70 per cent in 20 minutes (Fig. 3). Pectin Esterase No. 5 does not reduce the viscosity of pectin solutions (4). Therefore, beta-PG as well as alpha-PG must be produced by Fusarium because Fusarium PG hydrolyzes pectic acid more rapidly than pectin.

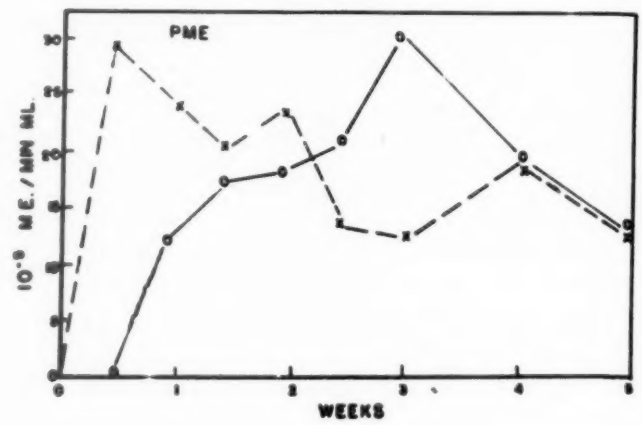
Product of PG action.—If the pectin of the host is hydrolyzed by Fusarium PM and the resulting pectic acid is not hydrolyzed by Fusarium PG, it is unlikely that the pectic materials will be liberated into vessels (see below). However, if the pectic acid is depolymerized to low weight molecules, the pectic substances will be liberated, but no gel will form to obstruct flow. If, on the other hand, intermediate weight molecules accumulate, calcium pectate gels will form. Therefore, the nature of the product of the action of Fusarium enzymes is of importance.

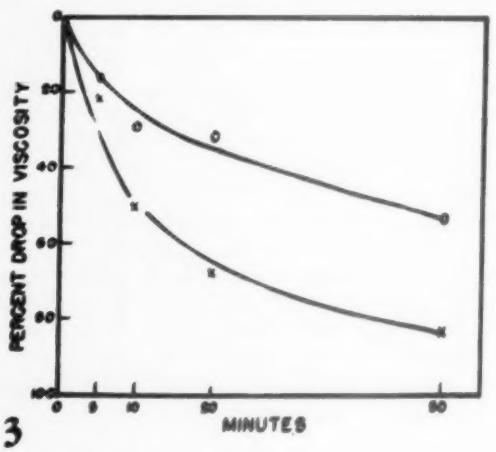
Because Fusarium grew on pectin as a sole carbon source, it evidently hydrolyzed pectin to galacturonic acid. Further evidence on this matter was sought. In accordance with methods outlined by Dingle et al (8), a 16-day Fusarium filtrate from pectin medium was examined. Because no gel formed when 1 volume of 95 per cent ethanol, 1 volume of 5 N HCl, or 1 volume of 10 per cent $CaCl_2$ was added to aliquots of the filtrate, the product must be of very small size.











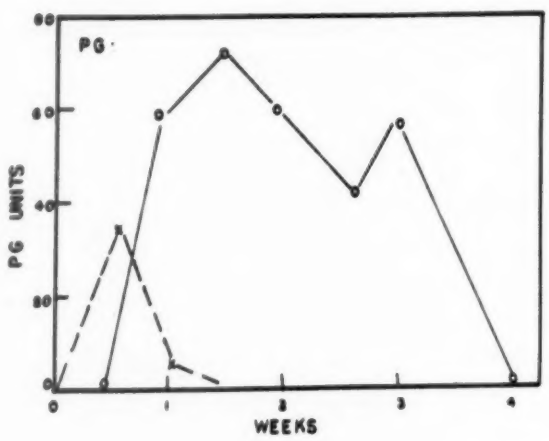


FIG. 1-4. Fig. 1. Diameter of zone of pectic acid hydrolyzed by Pectinol 100D (x) and filtrate from Fusarium culture filtrate (o). Fig. 2. M.e./ml/minute of ester bonds hydrolyzed by PM from Fusarium culture filtrate. Fig. 3. Change in viscosity of a pectin solution caused by a Fusarium PG preparation low in PM with (x) and without (o) PM added. Fig. 4. Dry weight of Fusarium mycellium and PM and PG concentration in pectin shake (x) and quiet (o) cultures.

However, no monogalacturonic acid accumulated, as shown by the failure of basic lead acetate precipitate to turn red when boiled (9). Evidently no polymers of galacturonic acid capable of forming gels will accumulate in the presence of the fungus.

Sixty ml of 2 per cent pectic acid with 4 ml of PG purified from Fusarium filtrate was incubated under toluene. After 48 or 66 hours galacturonic acid had appeared, as shown by the positive basic lead acetate test. The molarities of reducing groups of aliquots of the pectic acid-PG mixture were: 0 hours, 0.006; 18 hours, 0.057; 42 hours, 0.062; 66 hours, 0.062. When a sample was autoclaved in 0.5 N HCl for 30 minutes the molarity of reducing groups was 0.114. These values indicate that galacturonic acid is produced by PG from Fusarium.

Although pectins are evidently hydrolyzed by Fusarium to molecules that will not form gels, the supply of host pectin is not limiting and molecules sufficiently large to form calcium pectate gels may occur during the continuous hydrolysis and may be numerous enough to obstruct flow. Another possibility is that galacturonic acid may be formed into obstructing gums by some process not yet understood (17, 30).

PRODUCTION OF PECTIC ENZYMES BY FUSARIUM IN VITRO.—Fusarium grew rapidly on pectin medium, attaining maximum weight in 3 days in shake culture and in 10 days in quiet culture (Fig. 4). As growth occurred, the pH of the nutrient solution dropped from an initial value of pH 5 to pH 4 and then rose steadily until it levelled off at pH 8 after 14 days. In shake culture maximum yields of PG and PM were found on the third day when the first measurements were made. Thereafter PG decreased more rapidly than PM. As a consequence there was considerable PM but no PG on the tenth day. Thermal inactivation of these enzymes, reported above, accounts for their individual rates of disappearance from culture filtrates. Hence a shake culture on pectin medium is a quick source of PM and PG and, if the incubation period is extended, a source of PM practically free of PG. In subsequent experiments, Fusarium attained maximum weight in pectin shake culture in 1 day and enzymes were at a maximum in 2 days.

In quiet cultures, growth and enzyme production were slower (Fig. 4). Thus maximum yields of mycelium and of PG occurred on the tenth day, while PM continued to increase for 21 days. In quiet culture on a glucose medium, growth was less rapid than on pectin and PM was produced in the same manner as it was in a quiet pectin culture. In glucose-casein hydrolysate nutrient in shake culture PM yields were lower than in pectin medium but growth was rapid. Inasmuch as no PG was detected (plate or viscosity assay) in culture on either glucose medium, the production of PG depends upon the substrate whereas PM is produced on a nonpectin substrate.

The production of pectic enzymes by other fungi contrasts with that of *F. oxysporum* f. *lycopersici*. Production of both PM and PG by *Penicillium chrysogenum* depends upon the substrate (31). However, production of PM but not of PG depends upon substrate in *Botrytis cinerea* and *Aspergillus niger* (11, 12).

Various species of *Fusarium* have been shown to produce PM in bran culture (39). The reported concentrations of PM in water extracts of these cultures are much lower than are those shown in Figure 4. In the present study an 8-day culture of Fusarium on bran extracted by the procedure of Winstead and Walker (39) was compared in PM content with an 8-day pectin shake culture in which the same amount of inoculum had been used. The PM in the bran culture extracts and shake culture filtrates hydrolyzed 2.2 and 41.6×10^{-5} m.e./ml/minute, respectively. The former is the same order of magnitude of PM concentrations obtained by Winstead and Walker from bran culture. The bran and shake cultures provided 0.0029 and 0.048 units of PG, respectively. The results indicate that higher concentrations of pectic enzymes can be obtained in pectin culture filtrates than in water extracts of bran cultures.

ENZYME PRODUCTION IN VIVO.—Sections of Fusarium infected Bonny Best tomato stems were inspected for evidence of pectic enzyme action. None was found in moderately diseased plants. However, evidence of maceration was found in severely diseased plants, all leaves of which were abscised and in the vessels of which much mycelium was present. Several bundles were entirely deteriorated and appeared as holes surrounded by discolored parenchyma. Some vessels were filled with brown material. Other workers have observed dissolution of cells in the vicinity of severely infected xylem (25) and corrosion and swelling of infected vessels (35). It is possible that the deterioration is due to pectic enzymes and that the dark material is an indirect product of pectic enzyme activity (17).

A more specific test for the presence of enzymes in diseased plants would be to demonstrate their presence in the vascular sap exuded by root pressure from the stumps of diseased plants. To test for the presence of PM, 1 ml of sap from check plants and 1 ml from wilted plants inoculated 2 weeks previously were each mixed with 2 ml of a pectin, NaCl, and methyl red solution. The change in pH resulting from hydrolysis of ester groups, colorimetrically measured by change in optical density at 520 mμ, was 8×10^{-4} units/minute for the healthy and 24×10^{-4} for the diseased plant sample. The rate of change was constant for more than 30 minutes. In the pH range used, 0.001 m.e. of acid changed the optical density of the diseased plant assay solution 0.220 units and of the healthy plant assay solution 0.319 units. Thus, considering the differences in buffering capacities, 4–5 times as many ester bonds were hydrolyzed by PM in diseased– as in healthy–plant sap. In another experiment, PM was found to increase with the severity of symptoms.

Since both the host and pathogen produce PM, the differences in characteristics of the enzymes from the

2 sources were used to determine the source of the extra PM present in diseased plant sap. First, the 2 enzymes differ in their response to pH. That from the healthy host is much more active at pH 7 than at pH 5, whereas the enzyme from the pathogen is about equally active at the 2 pH values. By using bromthymol blue instead of methyl red the spectrophotometric assay was modified to estimate PM activity at pH 7. At pH 7 the activity in diseased plant sap was only 2–3 times as great as in healthy sap. That is, the healthy plant sap gained in activity relative to the diseased as the pH was raised. This was the behavior expected if the extra PM in the diseased plant was produced by the pathogen.

The PMs from the 2 sources also differ in response to wetting agents. PM from the host is inhibited and PM from the pathogen is not inhibited by Triton 720. When 0.1 ml of a 3 per cent solution of Triton was added to the 3 ml of assay solution, hydrolysis, as shown by change in the indicator color, continued in the solutions containing Fusarium PM or diseased-plant sap but slowed down or stopped in the solution containing PM from healthy tomato stems. This evidence, like the response to pH, indicates that the extra PM present in diseased plant sap is produced directly by the pathogen and not indirectly by its action on the host.

The presence of PG in diseased plant sap has not yet been established.

EFFECT OF ENZYMES UPON TOMATOES.—*Stem sections.*—A mixture of PM and PG would be expected to cause the dissolution of the middle lamella and, hence, the breakdown of tissue. However, it is not expected that action by PM alone could lead to the breakdown of tissues (2). To test these expectations 6 sections of Bonny Best tomato stems 1 cm long were split longitudinally and weighed into flasks containing 25 ml of raw or boiled 1 per cent solutions of Pectinol 100D (PG plus PM) or Pectin Esterase No. 5 (PM without PG). The enzyme solutions contained toluene and 0.05 M acetate buffer, pH 4.5. The mixtures were shaken for 1 day and the stem sections removed and weighed. The sections in the raw and boiled PM alone weighed 99 per cent of the initial weight, in the boiled PG plus PM, 98 per cent, and in the raw PG plus PM, 63 per cent. Only the epidermis and vascular system remained of the sections in the raw PG plus PM.

In a similar experiment stem sections were shaken for 3 days in buffer, toluene, and a filtrate from a 70–day old Fusarium culture which contained no measureable PG but sufficient PM to hydrolyze 6×10^{-5} m.e./ml/minute at 37°. Sections were also exposed to a Pectinol 100D solution containing about the same PM activity and also PG activity. The sections in Fusarium PM did not lose weight, but those in Pectinol 100D did. Hence, PG as well as PM must be present in diseased plants to account for the maceration observed. Further, if maceration is a step in the production of discoloration and plugging in Fusarium wilt, then PG, as well as the PM suggested by Gothoskar et al (14), must play a role.

Cuttings.—An inherent difficulty of experiments in which tomato cuttings are exposed to test solutions is the non-specificity of the response. Large molecules such as pectin, polysaccharides, or polyethylene glycol (18), or high concentrations of ions will cause tomato cuttings to wilt. High concentrations of protein, e.g. in an enzyme preparation, are expected to wilt cuttings because 0.45 mg/ml of dialyzed egg abumin will cause wilting of petiole or stem cuttings in 2 hours (midday, overcast sky, 25°C). Therefore, observations of wilting following exposure of cuttings are of limited value. These difficulties were recognized early (19).

Macroscopic observations of vascular discoloration cannot distinguish between browning of the xylem parenchyma and accumulation of brown material in vessel lumina. Although both phenomena occur in Fusarium wilt, their causes can be separated. Hence, microscopic examination of sections of exposed cuttings is necessary. Caution is also necessary in the interpretation of microscopic examinations because contaminating debris or phenols could cause plugs or discoloration.

In many experiments, petiole and stem cuttings have been exposed to dialyzed preparations from the Celite separation of 3-day shake pectin culture filtrates. Assays of the fractions used in a typical experiment are given in Table 3. Reference to Table 1 A shows that lots 0 and 2, the raw filtrate and the first phosphate eluate, usually contain more glucosidase than 1 and 3, the material passing through Celite and the first NaCl eluate.

Five ml of the preparations before and after heating to 60° for 30 minutes at pH 6 were mixed with 5 ml of water. Less than 1 per cent of the PM or PG remained active after the heating (Table 2), but less protein was precipitated than if the solutions had been boiled. Stem cuttings of Bonny Best tomatoes were placed in the solutions for 1 day in the greenhouse, then placed in the laboratory for 4 days. Petiole cuttings were treated in the same way. Checks were provided by cuttings placed in 10 ml of water. With the exception of lot 0, the raw filtrate, the solutions were taken up readily and water was added to prevent the plants running dry. No leaf necrosis resulted.

TABLE 3.—*Preparations by Celite method from 3 day shake, pectin cultures of Fusarium*

Lot	0 Raw filtrate, turbid, blue	1 Through Celite, slightly turbid	2 Na_2HPO_4 eluate, clear	3 NaCl eluate, clear
Mg protein/ml	≧0.09	0.07	0.14	0.03
PM, %	25×10^{-5} m.e./ml/minute = 100	0	12	140
PG, %[a]	0.14u = 100	0	370	8

[a] Assay by cup plate.

On the fifth day, the action of pectolytic enzymes was evident: the butts of cuttings in unheated fractions were macerated to a height of 0.1–0.5 cm. Sections were made at a height of 1 cm. The raw filtrate, lot 0, produced black plugs in most vessel lumina. In the case of the stems these plugs were equally dark and numerous after heated lot 0 had been taken up. In the case of the petioles, black aggregates appeared on the butt ends of the vessels and the plugs were less numerous and dark in the lumina. The material passing through Celite, lot 1, was less turbid than lot 0 and produced no plugs in the stem cuttings. However, it did produce plugs in the petiole vessels. When lot 1 was heated, aggregates appeared on the butt ends of the vessels and the plugs decreased in number and darkness. A few plugs appeared in plants exposed to lot 2, high PG and low PM. Lot 3, high PM and low PG produced no plugs. In other experiments the darkness of the plugs has varied with the turbidity and color of the solutions used.

There was more browning of xylem parenchyma in plants exposed to unheated fractions than in those exposed to the heated fractions. However, no differences were discerned among the plants exposed to the raw fractions. This was true of other experiments that ran 5 days. In experiments that ran for shorter periods, fractions 0 and 2 produced more discoloration of xylem parenchyma than fractions 1 and 3.

Much of the area of the protoxylem was dissolved out 1 cm from the butt in the petioles exposed to lot 3, the fraction with high PM, low PG. Much less dissolution was caused by fraction 0, the raw filtrate. This was presumably due to the small amount of this material taken up. Stems were similarly affected but to a lesser degree. Heated fractions caused no dissolution.

In other experiments where the raw filtrate was less turbid and more was taken up, dissolution of xylem was observed. Also, when lot 2 contained considerable PM as well as high PG activity, dissolution occurred. On the other hand, when the PG activity of fraction 3 was extremely low, no dissolution was observed although PM activity was high. Apparently, both PM and PG were required for the dissolution of xylem just as they were for the rapid hydrolysis of pectin to material of lower viscosity as described above.

Low PG and PM concentrations were obtained by altering the culture medium. Filtrates from 3-day Fusarium cultures on glucose–casamino acids and on pectin media were dialyzed and subjected to a Celite purification. Enzyme and protein concentrations of the preparations from pectin medium were distributed in the usual manner and produced the usual effects upon cuttings exposed for 5 days. Protein contents of the fractions from casamino acids medium were about the same as for the pectin medium. However, no PG was present. The PM concentration of the casamino acids preparations was less than 1 per cent of that in the pectin preparations. The casamino acids preparations did not cause maceration of cutting butts or vascular tissue. The raw casamino acids culture filtrate and material that passed through Celite[4] caused more vascular discoloration than the fraction from pectin culture filtrate that was eluted by NaCl. This NaCl elution was capable of hydrolyzing 15×10^{-5} m.e./ml/minute of methyl ester linkages as compared to 0.13×10^{-5} for the raw casamino acid culture filtrate. There was less vascular discoloration in heated than in raw preparations. Thus, heat labile materials in the filtrate besides pectic enzymes produced discoloration.

From the foregoing observations several inferences were drawn. First, the plugs were not caused by enzymatic action but by the accumulation of material present in the solutions taken up by the cuttings. Second, parenchyma browning was caused by enzymatic action. The browning that occurred in less than 5 days was greatest in lots 0 and 2 (Table 3), high PG and glucosidase. The cause of browning was inactivated at 60°C, but glucosidase was not inactivated to any great extent at this temperature. Therefore, glucosidase was probably not the heat labile cause of discoloration. PG was inactivated at this temperature, but these experiments should not be taken as proof that PG was the sole cause of browning because other heat labile materials were undoubtedly present in lots 0 and 2. For example, the role of the heat-labile vascular browning entity, vasinfuscarin (13) in our studies was unknown. Further, these results did not exclude the possibility that PG and glucosidase together are a cause of browning.

Third, PM apparently did not directly cause browning of vascular parenchyma because fractions high in PM caused less browning in cuttings in 3–4 days than the fractions high in PG and glucosidase. In these experiments dealing with PM produced by Fusarium, the concentration was varied through purification and choice of substrate. There was no correlation of PM content with vascular browning. This result is at variance with the correlation observed when activity was varied by using different commercial enzymes or by growing different fungi on different media (14, 39).

However, fractions high in PM and also containing PG macerated xylem parenchyma. Therefore, PM was evidently limiting in this maceration just as it was in the reduction of the viscosity of citrus pectin. This maceration undoubtedly resulted in the union of tomato glycosidases and glycosides with consequent browning of parenchyma. This is thought to be the reason fractions high in PM and containing some PG led to parenchyma browning if cuttings were exposed for 5 days. In this sense, PM may be thought of as a contributing cause of parenchyma browning.

Discussion.—The above results show that the PG, which is produced by Fusarium growing on pectin but

[4] A heat stable material present in the casamino acids culture filtrate and not adsorbed by Celite caused leaf necrosis. No leaf necrosis was produced by pectin culture filtrates.

not on glucose, has the stability, pH optimum, and substrate specificity of other fungal PG. PM, on the other hand, is produced by Fusarium in measurable quantities on nonpectic medium but at much lower levels than on pectic medium. Further, Fusarium PM has a characteristic unusual for fungal PM; it is relatively active at pH 7. Its broad pH optimum suggests that more than one enzyme is present, although we have been unable to demonstrate this by affecting a separation.

The primary point of the present studies is the demonstration of the presence of *fungal* PM in the vascular stream of diseased plants. PG is also shown to be present probably. Because substrate is present and inhibitors are absent in this region where the pH is near the optimum for both enzymes, it is highly probable that hydrolysis will occur. Therefore, any hypothesis concerning the manner in which Fusarium causes tomato plants to wilt and their vascular bundles to become discolored must take into account the maceration of cells in the xylem and the appearance in the vascular stream for at least a short time of the maceration products.

A resonable hypothesis can be constructed. PG and PM produced by the pathogen macerate the cells of the xylem. Debris and some calcium uronide gels lodge in the vessels, particularly the small ones of the lowest petioles (7), and cause obstruction to flow. Such obstructions were early demonstrated in Fusarium infected cabbage stems (29) and recently in Fusarium infected tomato stems (25) and petioles (35). These obstructions cause a water shortage and wilting in lower leaves followed by wilting of upper leaves.

At the same time the maceration frees phenolic glycosides, which are then hydrolyzed by fungal enzymes or by host enzymes freed by the maceration.[5] The resulting phenols move into xylem parenchyma, are oxidized, and produce discoloration. Or, the phenols are oxidized by enzymes freed into the vessels, and then the quinones or melanins become part of the obstructions in the vessels. Davis et al (5) have already demonstrated the presence of glucosidase and phenol in infected tomato vessels and suggested their role in producing vascular discoloration.

How far have we gone in testing this hypothesis? Modifications of Koch's postulates have been suggested as criteria for establishing the role of a mechanism in pathogenesis. Reproducible identification in the diseased plant, purification, and reproduction of at least a portion of the disease syndrome by placing the toxin in a healthy plant should be the minimum conditions satisfied before vivotoxicity is said to exist (6). Studies of mechanisms possibly involved in producing Fusarium wilt can be evaluated in the framework of these criteria.

Early workers who exposed tomato cuttings to Fusarium culture filtrates did not, as they generally recognized, satisfy any of the 3 criteria (19, 34, 37). Although pectic material and pectic enzymes have been suspected (14, 19, 39), none of the criteria, i.e., identification in the host, purification, or production of symptoms by the pure materials, have been satisfied.

The present studies identify PM and possibly PG in the host and begin the establishment of their role in Fusarium wilt. However, purification and exposure of the host to purified material have not been accomplished. On the assumption that the pectic enzymes produced by Fusarium *in vitro* are identical with those produced *in vivo*, some progress has been made by means of the Celite method. However, many contaminants must still exist, e.g. glucosidase. In view of the long period of exposure of the cuttings used by others (14, 39) and by ourselves, viable spores would be the most serious contaminants. Contaminating material may very well affect the tomato cuttings because these plants are known to be susceptible to many materials (37).

Therefore, the results of exposure of cuttings to preparations so far made cannot be said to constitute proof of the role of the pectic enzymes in pathogenesis. Rather, the incrimination of the fungal pectic enzymes must rest for the present upon their observation in the diseased plant and upon the attractiveness of the hypothesis concerning their action.

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[5] Incubation of tobacco leaves infiltrated with 1 per cent Pectinol 100D caused the release of glyoxylic acid reductase (40) into the ambient solution after 5 hours at room temperature. Therefore, we assume that maceration by pectic enzymes can free substrates or enzymes from cells.

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STREPTOMYCIN SULFATE FOR THE REDUCTION OF BACTERIAL SOFT ROT OF PACKAGED SPINACH [1]

Wilson L. Smith, Jr.[2]

SUMMARY

The effectiveness of streptomycin in reducing post-harvest bacterial soft rot of spinach leaves packaged in plastic bags was tested. Streptomycin in pre-harvest sprays or post-harvest dips reduced decay so that the treated leaves showed virtually no decay after 2 days at 70°F. The amount of decay in them after 3 days was less or the same as in the controls after 2 days. Post-harvest dips in streptomycin solutions gave slightly better decay control than the pre-harvest sprays. Bacterial decay progressed rapidly in the spinach not treated with streptomycin and within 2 days a relatively high percentage of the leaves were invaded. In 3 days the leaves were entirely decomposed. Both treated and non-treated spinach held 1 week at 32° decayed more rapidly when transferred to 70° than similar lots of spinach that were not previously held at the low temperature.

Among the organisms that cause post-harvest decay of vegetables are a group of Gram-negative species of bacteria commonly referred to as soft rot bacteria. The compact mass of succulent leaves of packaged leafy vegetables are especially subject to decay by soft rot bacteria, and it has been estimated that such produce may be destroyed within 24–48 hours at temperatures of 70°F and above (12). Few studies on control of bacterial decay of packaged leafy vegetables have been made, and most have been concerned with the use of chlorinated wash water for reduction of decay of spinach (8, 10). Such treatments were relaitvely ineffective. At present, the best method known to delay bacterial decay of packaged leafy vegetables is to refrigerate at 40° and below. A supplemental treatment that would protect the vegetables from decay for 24 hours at 70° and above would be desirable because in many instances adequately controlled low temperature refrigeration is not available.

Recently, various authors (1, 2, 3, 4, 5, 6, 7, 9, 11) reported that antibiotics are highly toxic to a relatively large number of plant pathogenic bacteria and give effective disease reduction. Most of these studies were concerned with control of bacterial diseases of growing plants by antibiotics, the effect of the antibiotics on soil microflora, or the toxic action of antibiotics on micro-organisms in culture. Since these materials were shown to control bacterial diseases of growing plants it seemed reasonable that they may also reduce bacterial decay of plants after harvest. Preliminary tests in culture showed that the 2 bacterial species that commonly cause decay of harvested vegetables, namely *Erwinia carotovora* (Jones) Holland, *E. atroseptica* (van Hall) Jennison were extremely sensitive to a dilute concentration of streptomycin sulfate. The present report is concerned with the use of this antibiotic, hereinafter called streptomycin, for the reduction of bacterial decay of packaged spinach. A preliminary report of this work was given in 1952 (13).

METHODS.—Spinach for the experiments was obtained near Norfolk, Virginia, and in Baltimore County, Maryland. Treatments consisted of pre-harvest sprays and post-harvest dips. In the pre-harvest treatments spinach leaves were sprayed 5 days or 1 day before harvest with water containing 1000 ppm streptomycin in 0.5 per cent Tween 20. Similar spinach sprayed with 0.5 per cent Tween 20 served as a check. After harvest the sprayed leaves of each treatment or control were washed momentarily in 4 liters of water to which had been added bacteria washed from 2 each of 48 hour beef-peptone agar slant cultures of *Erwinia carotovora* and *E. atroseptica*. The post-harvest treatments consisted of a momentary, a 5-minute, or a 10-minute dip of spinach in water containing 1000 ppm streptomycin in 0.5 per cent Tween 20 and pure culture bacteria added in the same rate as in the wash water of pre-harvest sprays. Plate cultures in 1 test made after the momentary dip of spinach in the untreated water showed 11×10^6 viable bacterial cells. Cultures made from the momentary dip in the streptomycin solution showed 2×10^4 viable bacterial cells. Then the leaves of each treatment were whirled separately in cheesecloth bags to remove the excess water. Finally they were packaged in approximately 1-lb. lots in ventilated moisture-proof 450 MSAT cellophane bags and placed at 70°F.

Holding tests with spinach given pre- and post-harvest treatments were conducted 4 times, each replicate consisting of 4 bags. Half of the leaves sprayed in the field were washed, packaged, and placed at 70°F as soon as possible after harvest. The others were held at 32° for 1 week and then washed, packaged, and placed at 70°. Similarly, half of the freshly harvested untreated spinach was used in dip treatments immediately after harvest, and the other half was held at 32° for a week before dip treatments.

Daily readings were made during the holding period at 70°F until the spinach was entirely decomposed. The severity of decay was rated on the following scale,

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TABLE 1.—*Average decay ratings of water- and streptomycin-treated spinach packaged and held at 70°F*

Treatment	After 2 days [a]		After 3 days [b]	
	Water	Streptomycin	Water	Streptomycin
Pre-harvest spray:				
1 day before harvest	2.4	0.9	4.1	2.8
5 days before harvest	3.0	1.2	5.4	3.0
Post-harvest dip:				
Momentary	2.1	0.0	4.0	1.3
5-minute	3.4	0.2	5.8	2.0
10-minute	1.1	0.1	4.1	1.6

[a] L.S.D. 5 percent 1.91
1 percent 2.58
[b] L.S.D. 5 percent 1.75
1 percent 2.37

based on the approximate percentage decay of the external leaves in each bag: 0) no decay; 1) 1 per cent decay; 2) 5 per cent decay; 3) 20 per cent decay; 4) 40 per cent decay; 5) 60 per cent decay; 6) 80 per cent decay; and 7) between 80 and 100 per cent decay.

RESULTS.—After 2 days at 70°F, decay of the spinach not treated with streptomycin had progressed until all except that receiving the 10-minute water dip had an average rating higher than 2 (Table 1). Decay was decidedly reduced in all spinach treated with streptomycin. Spinach sprayed 5 days before harvest showed more decay than the other streptomycin-treated lots, with an average rating of 1.2. No decay had developed in 2 days in the spinach treated with the momentary post-harvest antibiotic dip.

The development of decay was rapid after an additional day at 70°F. All the bags of spinach that did not receive the antibiotic treatment had an average decay rating of 4 or above, indicating that a large number of the leaves were invaded by bacteria and that many of them were entirely decomposed. On the other hand, the highest rating of the spinach receiving the antibiotic treatment was 3.0 for that receiving 5-day pre-harvest spray, and the lowest was 1.3 for spinach receiving the momentary post-harvest dip. Decay in streptomycin-treated spinach after 3 days at 70°, therefore, was the same as or slightly less than that in spinach without streptomycin treatment at the end of 2 days.

Both pre- and post-harvest methods of application of the antibiotic are effective in reducing bacterial decay. The post-harvest dips consistently gave somewhat better, though not significantly better, decay control than the pre-harvest sprays. The fact that the fields were flooded by rain after application of the pre-harvest sprays may in part explain the difference. That decay control was obtained even though the spinach was covered with water in the field would indicate that a considerable portion of the antibiotic may have been absorbed by the spinach leaves.

Within 2 or 3 days, the decay ratings of spinach held for 1 week at 32°F before packaging and placed at 70° were almost always higher than those of similar spinach packaged immediately after harvest and placed at 70° (Table 2). The effect of the previous low-temperature holding period is especially apparent in the water-treated spinach and may indicate that the low temperature injured the leaves or predisposed them to decay. Pre-harvest streptomycin sprays followed by the cold period caused a considerable decay reduction for 2 days, but were relatively ineffective in 3 days. The post-harvest antibiotic dips of the chilled spinach gave effective decay reduction after 2 and 3 days at 70°. With both types of antibiotic treatments there was an increase in decay of the chilled spinach in comparison with the spinach packaged and put at 70° immediately after harvest, the differences being especially noticeable after 3 days at 70°.

DISCUSSION.—The tests reported indicate that streptomycin may be highly toxic to bacteria of the soft rot group and will protect spinach leaves from decay by these organisms for several days during the storage and marketing period. Since streptomycin is effective in reducing decay of spinach caused by soft rot bacteria, possibly it may be effective in reducing bacterial decay of other vegetables. Food materials should not be treated with this or other antibiotics until the effect of such treated food on the consumers has been adequately investigated and the use of antibiotics has been approved by the Food and Drug Administration.

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TABLE 2.—*Average decay ratings of water- and streptomycin-treated spinach packaged and placed at 70°F immediately in comparison with those of spinach stored 1 week at 32° before packaging and placing at 70°*

Treatment	After 2 days				After 3 days			
	Water		Streptomycin		Water		Streptomycin	
	70° immediately	32° followed by 70°	70° immediately	32° followed by 70°	70° immediately	32° followed by 70°	70° immediately	32° followed by 70°
Pre-harvest spray:								
1 day before harvest	1.0	4.2	0.2	1.6	2.8	5.5	0.8	4.8
5 days before harvest	1.9	4.1	0.4	2.0	4.0	6.8	1.9	4.1
Post-harvest dip:								
Momentary	1.9	2.2	0.0	0.0	3.0	4.9	1.0	1.5
5-minute	3.1	3.6	0.1	0.3	4.8	6.8	1.4	2.5
10-minute	0.4	1.9	0.1	0.1	3.5	4.6	0.9	2.3

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LISTING OF BOOKS

Phytopathology will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

Kamat, M. N. 1953. **Practical plant pathology.** Prakash Publishing House, Poona, India. 200 p. 65 plates. Price 20 s.

Written for the undergraduate student, this text presents a series of exercises designed to acquaint the student with the various classes of plant diseases and methods for studying them and their control. The book begins with 2 sections on methods; follows with sections on diseases caused by phycomycetes, Ascomycetes, basidiomycetes, fungi-imperfecti, bacteria, viruses, and miscellaneous causes; and ends with 7 appendices that discuss principles, taxonomy, and laboratory technique.

Childers, N. F., ed. 1954. **Mineral nutrition of fruit crops.** Horticultural Publications, Rutgers University, New Brunswick, New Jersey. 907 p. Illustrated. Price $10.00.

Arranged so that it can be used for a full college course in the nutrition of fruit crops, the book contains 18 chapters written by international authorities in specific fields. The first 14 chapters consider the major fruit crops of the United States; the last 4 chapters deal with isotopic tracers, chelated metals, experimental techniques, and mineral content; and a special photographic section of 134 pages illustrates the major element deficiencies. Plant pathologists will be especially interested by the attention given to various sorts of nutritional disorders.

Chupp, C. 1954. **A monograph of the fungus genus Cercospora.**

Charles Chupp, Ithaca, N. Y. 667 p. Illustrated. $10.00.

This book begins by discussing the characters that delimit the genus *Cercospora* and defining the taxonomic criteria employed in separating the species. It lists 1900 species according to the principal hosts attacked and describes them fully, the hosts being arranged alphabetically by families. Latin descriptions of many new species, and a number of new combinations, are given. Complete indexes of hosts and species of *Cercospora* are appended.

INCIDENCE OF CHARCOAL ROT OF POTATO IN BIHAR (INDIA) IN RELATION TO CULTURAL CONDITIONS [1]

M. J. Thirumalachar [2]

SUMMARY

Charcoal rot incited by *Botryodiplodia phaseoli* is a major disease of potato in Bihar, an important potato growing region in India. The losses range from 10–70 per cent, depending upon the susceptibility of the variety and predisposing conditions. The fungus gains entrance into the tuber from soil chiefly through proliferated lenticels. There is no secondary spread of charcoal rot from diseased to healthy tubers during storage.

Since the fungus grows rapidly at warm temperatures, cultural operations to reduce disease incidence were studied. Potatoes are grown during October to March in Bihar. Early planting by the middle of October and early harvesting after 110 days for Darjeeling Red Round and 140 days for Phulwa reduced disease incidence and yielded a good crop. Prolonged storage of tubers in the soil after the soil temperature has risen to 32°C and above, predisposes tubers to charcoal rot infection.

In the case of the late planted katwa crop (tubers are cut and planted by the third week of November), early harvesting after 90 days' growth prevents heavy disease incidence. More irrigations after the second week of February in the late planted katwa crop has been found beneficial in the case of the Darjeeling Red Round variety, possibly by reducing soil temperature and linear growth of the fungus in soil.

The charcoal rot disease of potato incited by *Botryodiplodia phaseoli* (Maubl.) Thirumalachar (*Macrophomina phaseoli* (Maubl.) Ashby) is a soil inhabiting fungus attacking the tubers and haulms under favorable conditions for the fungus. In India, the disease is widely distributed in Bihar and Uttar Pradesh states and has also been observed in potato samples received from West Bengal, Orissa, and Bombay states. The extensive storage rots reported in western India by Ajrekar and Ranadive (1) and in Madhyapradesh by Dastur (2) as being due to *Rhizoctonia destruens* may perhaps be the same disease.

Charcoal rot is of considerable economic importance and may inflict severe loss to potato during storage. Considerable losses have been reported due to charcoal rot in Palestine (4), Texas (10), Oklahoma (3), and other places. Littauer (4), for instance, stated that at the time of lifting tubers, the loss may only be 5 per cent, but generally may increase to 50 per cent in storage. Watson (10) in Texas stated that the loss was from 15–20 per cent, but some growers lost more than 50 per cent. Similar heavy losses, from 10–70 per cent, due to charcoal rot have been observed in Bihar, depending upon the period of harvest and presence of predisposing conditions.

DISEASE SYMPTOMS.—The disease symptoms have already been well described by Reichert and Hellinger (6), Littauer (4) and others. On tubers, the symptoms may either be of charcoal rot type involving blackening of lenticels and killing of eyes, or dry rot type when black sunken areas develop on the tuber surface. The latter type of infection is often mistaken for dry rot incited by *Fusarium coeruleum* (Lib.) Sacc. The fungus also incites stem blight under warm weather conditions producing often the pycnidial stage of *Botryodiplodia phaseoli* (7).

CAUSAL ORGANISM.—The fungus is isolated easily from diseased tuber tissue on potato dextrose agar. The growth is very rapid when cultures are incubated at 30°C producing greyish-white fluffy mycelia, which are pro-geotrophic in growth responses. Isolations were also made by transferring aseptically single pycnospores. As described previously (7) there are 2 cultural types in these isolations, one of them resembling the subspecies *typica* and the other, subspecies *sesamica* described by Reichert and Hellinger (6). Since the pycnidia and pycnospores produced by these 2 subspecies when inoculated onto potato stem are morphologically identical, the chief differences between the 2 subspecies lie in the type of growth on agar media and sclerotial development. It seems feasible to consider the 2 as varieties of *Botryodiplodia phaseoli;* and the names *B. phaseoli* var. *typica* Reichert & Hellinger and *B. phaseoli* var. *sesamica* Reichert & Hellinger are proposed.

INOCULATION EXPERIMENTS.—Some of the details of inoculation studies have already been given in connection with the testing of potato tuber varieties for charcoal rot resistance (8). It was shown that mycelia from 24– to 48–hour old cultures would produce charcoal rot within 5 days in wounded tubers kept at 28°C. Littauer (4) failed to obtain successful infection unless the tubers were previously subjected to moist heat of 55°C for 2 hours. The failure to obtain infection may be due to the use of old mycelium for inoculation, which on account of its evanescent nature breaks down, forming sclerotial bodies.

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The writer wishes to acknowledge his deep debt of gratitude to Professor Dr. I. Reichert, Professor of Plant Pathology, Agricultural Research Station, Israel, for personally discussing several of the problems of charcoal rot and giving benefit of valuable suggestions, and to Dr. S. Ramanujam, Director, Central Potato Research Institute for valuable suggestions and encouragement.

For studying the mode of penetration of the fungus within the tuber, mycelial fragments with sclerotia of *B. phaseoli* were incorporated in sterile soil in pots and surface sterilized tubers were buried. The soil was made moist and kept exposed to 28-37°C in the field. The tubers were taken out after 15 days and examined microscopically for penetration of hyphae. The strands of hyphae regenerating from the sclerotia used as inoculum were found firmly adpressed on the tuber surface. The lenticels had proliferated due to moisture, and several of them had been penetrated by the hyphae of *B. phaseoli*.

Charcoal rot control experiments.—In the absence of any commercial variety of potato with a high degree of resistance to charcoal rot, studies were made on control of the disease by cultural operations. Previous observations in Bihar as well as published data of other investigators had indicated that early harvested crops suffered less from charcoal rot than late harvested ones. Since the fungus is favored by warm temperature conditions for its growth, the effects of cultural operations such as early planting, early harvesting, frequent irrigations to reduce soil temperature, etc., were investigated.

a) *Time of sowing in relation to charcoal rot incidence.*—In the north Indian plains, potatoes are cultivated between October and March, the actual time of planting and harvesting varying from year to year depending upon the seasonal conditions. Experiments were laid out at the Central Potato Research Institute farm at Patna, in plots where the soil was known to be heavily infested with the charcoal rot inciting organism. Phulwa and Darjeeling Red Round varieties (which are chiefly cultivated in the plains) were chosen and planted on 4 dates at 14 day intervals starting from October 17, 1950. Three rows of 10 tubers replicated twice were planted for each planting date. The crop was harvested at maturity at 14 day intervals commencing from March 1, 1951, in the same order in which sowing was done. Soon after harvest, the tubers were stored at room temperature and examined at periodic intervals for disease symptoms and the number of charcoal rot affected tubers were counted.

TABLE 1.—*Charcoal rot incidence in relation to time of sowing*

Variety[a]	Date of planting	Date of harvest	No. of tubers stored	Percentage diseased
D.R.R.	10-17-1950	3- 1-1951	1442	20%
	10-31-1950	3-15-1951	1156	33%
	11-14-1950	3-29-1951	1040	40%
	11-29-1950	4-15-1951	986	53%
Phulwa	10-17-1950	3- 1-1951	1300	16%
	10-31-1950	3-15-1951	1618	19%
	11-14-1950	3-29-1951	1093	25%
	11-29-1950	4-15-1951	1400	30%

[a] Darjeeling Red Round is more susceptible than Phulwa to charcoal rot. Late planting in Phulwa produces numerous small tubers many of which could not be counted during harvest.

Many of the tubers that appeared apparently healthy at the time of harvest showed a gradual build-up of disease during the storage period. Tuber infection takes place only in the field and there is no spread of charcoal rot from diseased to healthy tubers during storage. The data on charcoal rot incidence in tubers harvested from 4 different dates of planting was calculated at the end of the storage season (the storage season is between April and September) and is presented in Table 1.

From the data, it is evident that in the early planted and early harvested crop charcoal rot incidence is less than in the late harvested crop.

b) *Early harvesting in late planted crop in relation to charcoal rot.*—Darjeeling Red Round is grown as a late planted crop (khatwa crop) in the plains for producing seed tubers for the next planting in the ensuing season. Tubers for this khatwa crop planting are imported from mid-hills in Nepal, cut and planted during November. The cool weather during this season of the year prevents rotting of cut tubers when planted. On account of late planting, harvesting is also delayed, with the result that tubers are exposed to the warm weather of the advancing summer season. Charcoal rot incidence in this crop is quite heavy. Experiments were made to find out the effects of early harvesting in this late planted crop with a view to study charcoal rot incidence in tubers. Sixty rows of 10 tubers each were planted on November 20, 1950 and the usual cultural operations were carried out. Starting from February 16, 1951 (93 days after planting) weekly harvests of 5 rows selected at random were made. The tubers of each lot were stored separately and observations made periodically during the storage season from April to September. Data on charcoal rot incidence in the different lots are presented in Table 2. All the haulms were dead by April 7, and harvests later than April 15 were not taken into account.

The results indicate that early harvesting of plants, after 90 days growth, keeps down the disease incidence. Delayed harvest will increase the percentage of disease without a significant increase in yield of tubers. Soil temperature above 32°C greatly favors the growth of the charcoal rot inciting fungus and also predisposes

TABLE 2.—*Charcoal rot incidence in relation to time of harvest. (Variety D.R.R.)*

Date of harvest[a]	Weight of tubers lb. oz.	No. of tubers stored	Percentage diseased
2-16-1951	37-0	1181	11
2-24-1951	41-4	1224	21
3-3-1951	39-0	1181	17
3-11-1951	47-4	1357	20
3-18-1951	43-8	1236	36
3-24-1951	50-4	1547	29
3-31-1951	44-8	1365	39
4-7-1951	47-0	1433	73
4-15-1951	41-0	1494	78

[a] Date of planting November 20, 1950.

the tubers for infection. Early harvesting of tubers 15–20 days before actual maturity of plants will not affect the keeping quality of tubers but will reduce disease incidence.

c) *Number of irrigations in relation to charcoal rot in late planted crops.*—The effect of number of irrigations in the late planted crop in relation to disease incidence was investigated. Littauer (4), in giving general recommendations for the control of charcoal rot disease, suggested that more frequent irrigations tend to decrease disease incidence by keeping down soil temperature. Watson (10), on the other hand, stated that in Texas high soil temperature and moisture are the chief epiphytotic factors.

Experiments were made using Phulwa and Darjeeling Red Round varieties. The tubers were planted on December 9, 1950, and from February 16, 1951, onwards (when the noon soil temperature was as high as 30°C), one set of Phulwa and D.R.R. plots received 2 irrigations per week and the other only 1 irrigation. Eight rows of 10 plants each were planted for each treatment combination. The crop was harvested on April 16, 1951, and observations for charcoal rot incidence were recorded for the tuber lots during the storage period. The data are presented in Table 3.

Phulwa showed no differences in disease incidence in the 2 treatments whereas D.R.R. showed less disease incidence in the lot receiving 2 irrigations. This may be due to the fact that since Phulwa is more field resistant than D.R.R., the difference between the 2 treatments is not marked. Norton (5), working on charcoal rot on guayale, showed that *B. phaseoli* grows best at soil temperatures between 32 and 37°C and at 5 per cent soil moisture. Increase in percentage of soil moisture decreased the linear growth rate of the fungus. He attributed the increased disease incidence chiefly to weakening of the host under high temperature conditions.

TABLE 3.—*Incidence of charcoal rot in relation to number of irrigations*

Variety[a]	No. of irrigations (per week after 2-16-51)	No. of tubers (for 80 plants)	Percentage diseased
Phulwa	1	4826	20
	2	4920	19
D.R.R.	1	1874	57
	2	1656	37

[a] Date of planting 9-12-1950. Date of harvest 4-16-1951.

Effect of cold storage of tubers on charcoal rot incidence.—Tubers stored soon after harvest in the commercial refrigerated cold stores (5°C) remain firm and apparently healthy to the end of the storage season (September), even though many of the tubers contracted charcoal rot infection while in the field. The cold temperature prevents the spread of the fungus within the tuber and development of a total rot. However, when the tubers are taken out of cold storage by the end of September for planting and stored at room temperature, the fungus within becomes activated and brings about the rotting of tubers within 10 days. As a result of these observations it has been found advisable not to plant tubers immediately after taking them out of cold storage since many of the seed tubers may rot and result in poor stands. A period of 10–15 days incubation of tubers at room temperature would enable detection of charcoal rot affected tubers and their removal before planting.

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EQUIPMENT AND METHODS FOR THE ISOLATION OF PATHOGENS FROM CLOVER SEED[1]

Charles M. Leach

SUMMARY

An improved method of isolating pathogens from seed of small-seeded legumes utilizes special equipment designed to expedite the preparation and plating of both treated and untreated seed. The special equipment consists of a seed disinfecting apparatus, an aseptic seed drier, and 2 vacuum counters for plating seed.

While investigating seed-borne pathogens of small-seeded legumes, mainly the clovers, it was found that plating seed on 2 per cent malt agar is a suitable method for detecting and isolating pathogens. Plating untreated seed reveals the superficial organisms present on or in the seed coat but does not generally reveal those within the seed. This latter group of organisms is apt to be concealed by rapidly growing surface-borne fungi, unless the seed are surface disinfected. Such treating and plating of seed is very tedious by the usual procedures. To facilitate handling large samples of seed, the following special equipment was designed or adapted.

SEED DISINFECTING APPARATUS.—This apparatus (Fig. 1, A and B) is a modified version of that designed by H. W. Johnson[2] and is used with a mild disinfectant to eliminate the surface microflora of seed. The apparatus consists of:

a) A cylindrical copper disinfectant chamber, closed by means of a rubber stopper. There are 2 inlets at the top of the chamber. One leads to a copper tube which branches into a "Y." This serves as a feed for disinfectant and sterile distilled water and is connected by rubber or plastic tubing to 2 supply flasks. The other inlet leads to a "T" shaped copper tube which bears an air filter on the upper arm of the "T." This allows the passage of spore-free air into the chamber. A vacuum may be applied to the disinfecting chamber through the lower arm of the "T" to remove air from the seed. At the bottom of the chamber is an outlet through which disinfectant and water can be drained.

b) Two wire mesh seed containers (Fig. 1, C) which fit one above the other in the disinfecting chamber and allow 2 different seed samples to be treated simultaneously.

ASEPTIC SEED DRIER.—Seed must be dry if vacuum counters are to be utilized in plating. An aseptic seed drier (Fig. 2, A and B) is used to dry the moist disinfected seed after its removal from the disinfecting apparatus. The drier consists of an air blower, a glass wool air filter, and a drying chamber. The drying chamber portion of the apparatus is detachable from the blower and can be sterilized in an autoclave. The 2 wire mesh seed containers as used in the disinfecting apparatus fit one above the other in the drier. They may be removed by means of a hooked metal rod.

VACUUM SEED COUNTERS.—Seed plated on an agar substrate must be well separated, otherwise the outgrowing fungi merge before they can be observed and isolated. By using vacuum counters and taking care to prevent contamination, a definite number of seed may be picked up quickly, then deposited well spaced on agar plates (Fig. 4). The vacuum counters (Fig. 3, A and B) may be successfully operated from the vacuum produced by a filter pump attached to a faucet. The plate type (Fig. 3, B) is similar to the counters used for germination tests in many seed testing laboratories. It differs in that it picks up fewer seed and has a simple mechanism for breaking the vacuum. The vacuum may be quickly broken by raising the index finger from the hole at the end of the handle. The claw type pickup (Fig. 3, A) is cheaper to construct and is more suitable for larger seed.

OUTLINE OF THE PROCEDURE USED TO EXAMINE SEED.—The sample to be examined is divided into 2 portions. One portion is plated untreated with the vacuum counter to determine whether surface-borne pathogens are present (Fig. 5, A). The other portion is surface disinfected and then plated to determine the pathogens present within the seed (Fig. 5, B and C). The seed to be surface disinfected are placed in the container (Fig. 1, C) and inserted into the disinfecting apparatus (Fig. 1, A and B). The disinfectant, composed of a mixture of 4 parts 5.25 per cent sodium hypochlorite, 1 part 95 per cent ethyl alcohol, and 8 parts distilled water, is run into the disinfecting chamber for a predetermined period of time. With seed that are difficult to surface disinfect, a vacuum may be applied at this stage. The disinfectant is drained off and the seed washed several times with sterile distilled water. The seed containers are then removed by means of a hooked rod and placed in the chamber of the drier (Fig. 2, A and B). The blower is started and run until the seed is dry. The seed containers are then removed,

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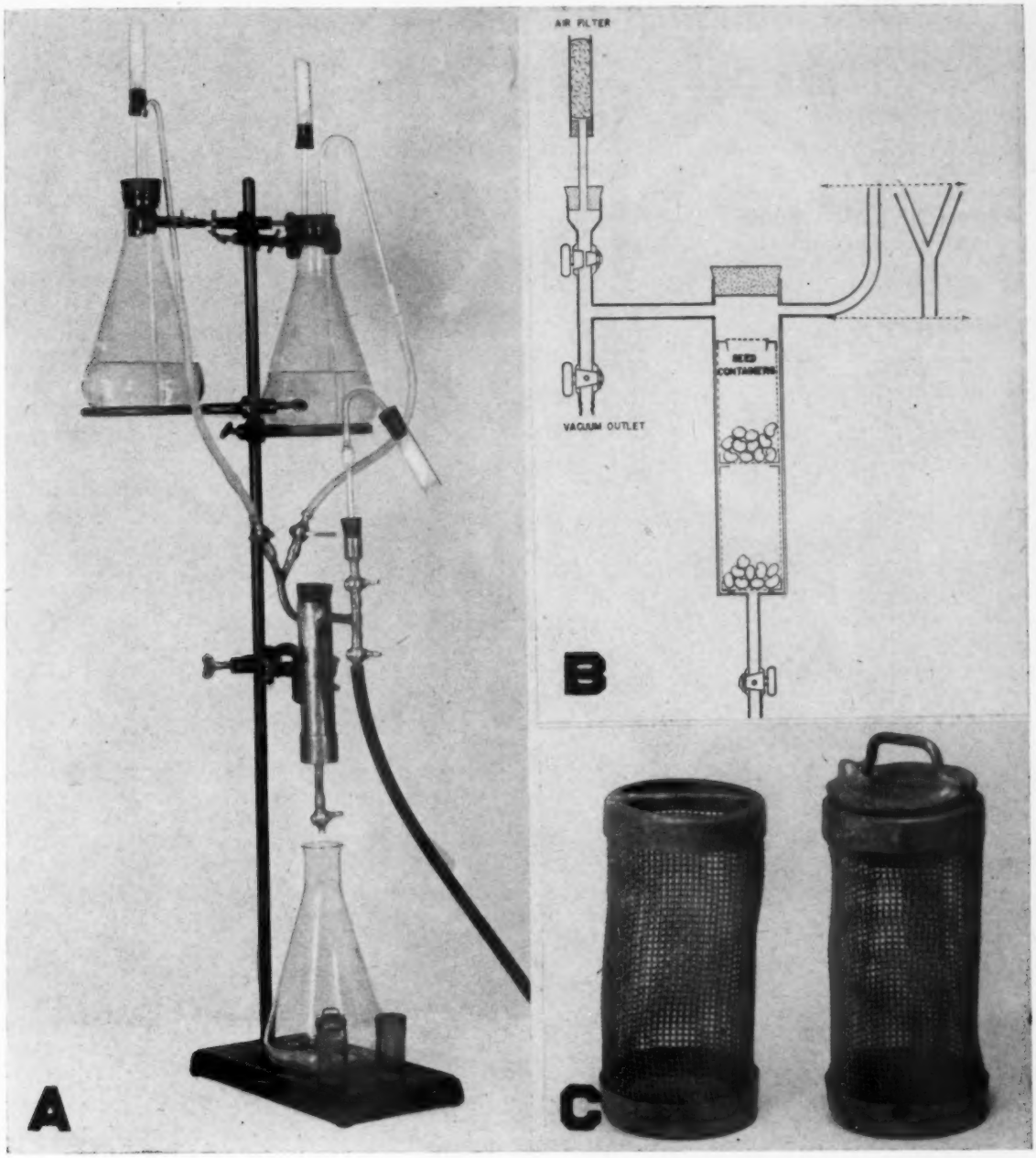

FIG. 1. Seed disinfecting apparatus. A) Complete apparatus. B) Sectional drawing through disinfecting chamber. C) Seed containers.

and emptied into sterilized Petri dishes. The dried surface-disinfected seed can then be plated out on malt agar with a vacuum counter.

With the plate type counter, seed are placed on the suction plate with the vacuum on. The counter is then inverted to remove excess seed and held over an open Petri dish with the suction plate near the agar substrate (Fig. 4). The vacuum is broken and the suction head is given a sharp tap to deposit the seed on the medium.

By using vacuum counters within a small inoculating chamber (approximately 2½ ft. × 2½ ft. × 2½

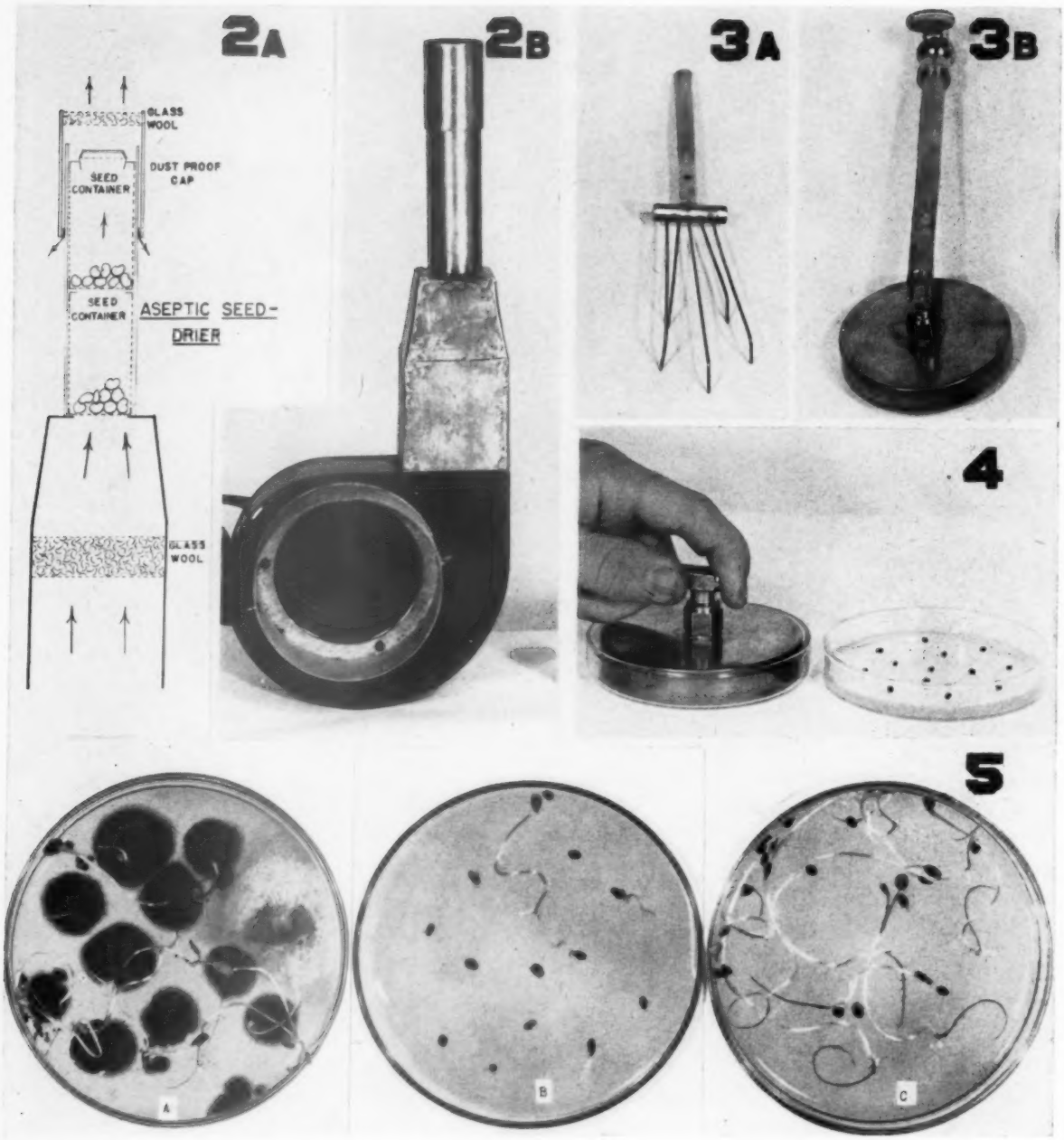

FIG. 2-5. Fig. 2. Aseptic seed drier. A) Sectional drawing through drier. B) Complete apparatus.—Fig. 3. Vacuum seed counters. A) Claw type. B) Plate type.—Fig. 4. Vacuum counter in use.—Fig. 5. Clover seed 7 days after plating. A) Nondisinfected. B and C) Surface disinfected.

ft.), this method of plating enables 800 dried clover seed (13 per plate) to be plated within 30 minutes with remarkably little contamination. With clover seed, it has been found that fungus colonies may usually be distinguished 7 days after plating on malt agar when incubated at 70°F.

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BARLEY ADAPTATION IN RELATION TO PYTHIUM ROOT ROT [1]

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SUMMARY

Early barleys yielded less and appeared to suffer more from root rot than did adapted varieties of medium to late maturity.

Poor performance was most common in barleys from Egypt, Afghanistan, and India, where the grain is usually fall sown. The long photoperiods following spring sowing in South Dakota caused the barleys from these countries to mature too early for optimum yields.

Some varieties responded strongly to photoperiodic treatment, and yields in the root-rot test plot were correlated with root and shoot development under controlled photoperiod conditions in sand-nutrient cultures and inoculation with *Pythium arrhenomanes*.

Early barley varieties yielded poorly in southcentral South Dakota in a soil heavily infested with root rot pathogens, especially *Pythium arrhenomanes* Drechs., while later maturing varieties, with some exceptions, were more productive (1). Klages found that early varieties were high ranking in certain seasons but that the variety Odessa yielded higher over a long period of years at Highmore (8), and Grafius and Dirks found that Odessa and Trebi outyielded 2 earlier varieties at the same location during a more recent period of years (5). The implication that later maturing varieties of barley are better adapted to the complex of environmental stresses of central and western South Dakota is contrary to the long established concept that early maturity is essential for successful barley production in this area. Further investigations of the influence of environmental conditions on the response of several contrasting genotypes in barley were therefore initiated. This paper reports on results of these field and greenhouse investigations; it deals with a few ecotypes with contrasting photoperiodic responses.

FIELD EXPERIMENTS.—The field experiments, in southcentral South Dakota, were in an area where production of barley, wheat, and similar grasses had become unprofitable, yet alfalfa produced well. The soil, a Huggins sandy clay type, was low in nitrogen and organic matter. The response of a limited number of barley varieties to nitrogen and partial soil sterilization with chloropicrin was studied from 1948 through 1950 (1). The response of a larger collection of barleys, representing a greater range of types, was studied in 1950 and especially in 1951. The data obtained suggested pertinent interactions between varieties, environment, and root rot in relation to yield of grain.

Since these studies were terminated following the 1952 experiment, the data cover only a short period of field experimentation. They suggested that early maturing varieties of barley responded less favorably in terms of grain yield to the environment and root and crown disease complex than later maturing varieties. A general study of climatic adaptation of barleys in South Dakota by Grafius and Dirks (5) indicated a similar varietal response. An analysis of the specific data from the root rot nursery suggested that ecologically different types accounted for part of the effect of early and later maturity upon yield.

Experiment 1.—Ninety-nine varieties were divided into 2 approximately equal groups and sown from April 10 to 13, 1951, in 2 blocks each containing 4 replicates, in a root rot nursery in southcentral South Dakota. Feebar, Minsturdi, Odessa, and Plains were grown in each block to permit direct comparisons. Heading notes were taken and converted to a maturity index ranging from 24, for varieties that were fully headed at an early date, to 0 for those showing no heads 10 days later. The center rows of each 3-row plot were threshed and the grain weighed. Growth differences among varieties were visible when elongation of the main culm had reached the flag leaf stage and persisted through all subsequent stages of growth.

Varietal differences are important in adaptability. The earliest trials (1) established a varietal response that was more or less repeated every year. Trebi and Odessa, considered tolerant to Pythium root rot, outyielded Plains and Feebar, rated susceptible, by 32 per cent, 31 per cent, 49 per cent, and 19 per cent in the seasons 1948-1951. Taking these figures as rough disease indices, root rot losses were severe in 1950 and light in 1951. Both seasons were cool but moisture was abundant in May and June of 1951 (10 in.) and meager in 1950 (3.9 in.). The data of the single trial of 1951, expanded to 99 entries, are not adequate to compare individual varieties, but some appeared better than others (among the early ones Hank, Biskra, and Club Mariout yielded better than Atsel, Olli, or Pannier). By considering groups of varieties, or classes, it is believed that the data are meaningful.

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Sincere thanks are due to John Pospisil of Colome, South Dakota, who cooperated in these investigations through the use of his land and facilities; to Mr. I. Bangdiwala of the Agricultural Experiment Station of the University of Puerto Rico for his aid in the more complicated calculations; and to J. G. Dickson, G. A. Wiebe, C. M. Nagel, and H. A. Rodenhiser for their assistance in the preparation of the manuscript.

FIG. 1. Medium late barley, H-35-7-2-1-3, (left); very early barley, Besert 13, (right) in the root rot nursery in 1951.

The strength of the association of maturity class and ability to yield and produce straw of desirable length under severe root rot conditions (Fig. 1) became obvious as the number of varieties under observation increased (Fig. 2). The curvilinear relationship of maturity class and yield indicated a best adapted mid-late class. The data of 1950 (24 varieties) and 1951 (48 varieties in block 1, 55 in block 2) were so similar in this respect that no significant differences existed, although the mean yields of the 3 sets of data were 10.3, 14.8, and 17.4 bushels per acre, respectively. The optimum calculated maturity class shifted (6.1, 6.7, 8.4, respectively) slightly toward earliness as the mean yields increased. Calculations of bushels of grain produced per day from planting to harvest gave 0.142 bushels per day in the extremely early group, 0.226 in the best intermediate class, and 0.219 in the latest class. The relation between height and maturity class was linear. Average heights ranged from 12.9 in. for the earliest group to 22.8 in. for the latest group.

Experiment 2.—Geographic source, as well as individual varietal differences and maturity class, may be an important factor in the adaptation of barley with respect to Pythium root rot. For example, in the 1951 test the Manchurian and Ethiopian types were generally superior to the introductions from Egypt and India. This led to a study of 370 entries in the world collection of barleys maintained by the U. S. Department of Agriculture.[3] These were tested in Pythium infested soil in 1952 by growing each entry in single, 7-foot rows, replicated 4 times. Relative adaptability was determined on the basis of plant height, those failing to attain sufficient height for easy mechanical harvest (about 8 in.) being classed as failures.

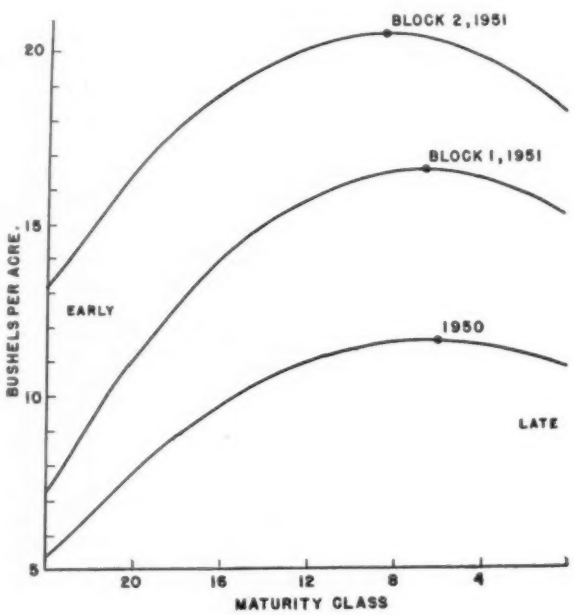

FIG. 2. Relation of rate of maturity to yield of barley in bushels per acre in the root rot nursery, 1950 and 1951. The calculated curvilinear means described the data more closely than linear means, indicating that there is a best adapted intermediate maturity class. The optimum calculated maturity class shifted slightly toward increased lateness as mean yields decreased.

[3] David J. Ward and G. A. Wiebe, Division of Cereal Crops and Diseases, U.S.D.A., kindly furnished the seed.

In this test, it was further evident that source is an important factor in barley adaptation. Again the Manchurian and Ethiopian barleys were superior to those from Egypt and India. Likewise the barleys from Iraq were good, but those from Algeria and Afghanistan were poor. By way of numerical comparison 97 per cent, 93 per cent, and 93 per cent of the barleys from Manchuria, Ethiopia, and Iraq, respectively, were acceptable, while only 32 per cent, 34 per cent, and 57 per cent, and 66 per cent of those from Egypt, Afghanistan, Algeria, and India, respectively, made adequate growth.

GREENHOUSE EXPERIMENTS.—In the main, barleys adapted to regions where the crop is fall sown, such as Egypt, failed in greatest numbers. In northern latitudes, winter culture exposes the plant to different photoperiod conditions than spring culture. Lack of adaptation to light conditions of spring sown crops in the northern plains was suggested as a predisposing factor acting to weaken those unadapted forms which responded strongly to photoperiod. Greenhouse tests with controlled photoperiods were conducted to investigate the possible role of these factors in influencing root rot losses in barley. Silica sand in 8-inch clay pots and a low phosphorus nutrient solution (4) were used in 3 experiments. Two photoperiods were used in each case, 18 and 9 hours of light in experiment 1, and 15 and 9 in experiments 2 and 3. Illumination with 300-watt Mazda bulbs extended the natural photoperiod, and cages covered for a part of each day with black, sanforized broadcloth, mercerized, (thread count 690) provided the short photoperiod. Experiment 1 ran from October 17 to December 17, 1951, and experiments 2 and 3 from January 6 to March 6, 1952. Experiments 1 and 2 were conducted at 70°F, and experiment 3 at 75° for the first month, and 70° for the second. The nutrient solution of experiment 1 was adjusted to pH 6.3 with NaOH, whereas those of experiment 2 and 3 were brought to pH 6.8. In 3 weeks after planting the lower leaves of barley in experiment 2 and 3 in the long photoperiod were bleaching. The addition of 360 ml of a molar solution of sodium chloride to each 10 liters of nutrient solution ameliorated this trouble.

Six-day-old cultures of *Pythium arrhenomanes* grown on Rands and Dopp's medium were hand-kneaded into the bottom 3 in. of sand. The contents of 1 Petri dish was used per pot. The same isolate was used in experiments 1 and 2, whereas a mixture of 4 isolates was used in experiment 3. The pots were then filled to within 3 in. of the top with noninfested sand. Seedlings were started 3 days in sterile sand from Arasan-treated seed and then transplanted and covered with clean sand. The pots were then watered with tap water. The nutrient treatment of 200-250 cc of dilute solution was started a week later and applied every 4 days. Tap water was added as needed between applications of nutrient solution; this insured flushing of the sand. Each pot contained 2 plants each of 2 varieties and there were 4 replications. A total of 16 varieties were tested. The differential light treatment began with emergence. Observations on plant characteristics were made during growth and at harvest. The dry weight of shoots and roots was recorded.

Experiment 1.—Differences between the sets of plants grown under the 2 light treatments were evident after about 24 days. The longer photoperiod produced narrow, grayish green leaves, thin culms, and early heading. Eleven heads of Atsel and 6 of Vaughn appeared 41 days after planting. All varieties headed in the longer photoperiod, while only Atsel and Vaughn headed in the shorter photoperiod. At the long photoperiod, 102 heads appeared on the 4 early varieties, but only 57 heads on the 4 late varieties (Table 1). The dry weight of shoots of Montcalm and Plush was favored by a long photoperiod, whereas Atsel and Vaughn showed an opposite effect.

A greater dry weight of roots was produced by exposure to the short photoperiod than to the long photoperiod, although the root system of late varieties was

TABLE 1.—*Heading, tillering, and dry weight of 8 barley varieties grown under 2 photoperiods*

Varieties	C.I. No.	Number of hours of light: 18 (No. of heads)	9 (No. of heads)	18 (No. of tillers)	9 (No. of tillers)	Dry wt. of shoots; Ratio long light/short light[a]
Early varieties						
Atsel	6250	24	21	15	0	0.81*
Vaughn	1367	32	4	29	6	0.87*
Olli	6251	23	0	0	6	0.95
Plains	7250	23	0	1	3	1.02
		102	25	45	15	
Late varieties						
Peatland	5267	18	0	1	8	1.07
Montcalm	7149	17	0	7	6	1.10
Vantage	7324	14	0	6	6	1.20*
Plush	6093	8	0	7	16	1.28**
		57	0	21	36	

[a] Significant departure from the 1:1 ratio; * 5 per cent leve l, ** 1 per cent level of significance.

TABLE 2.—*The effect of a 9- and 15-hour photoperiod on the development of 6 varieties of barley in a low phosphorus nutrient solution*

Variety	C. I. No.	Ratio Dry weight of shoots, 9 hr/15 hr. photoperiod	Ratio Dry weight of roots, 9 hr./15 hr. photoperiod	Ratio Elongation of main culm, 9 hr./15 hr. photoperiod	Total heads on 16 plants in boot with awns showing by March 15		Tillers per plant photoperiod		Leaves per main axis, 15. hr. photoperiod
					9 hr. day	15 hr. day	9 hr.	15 hr.	
Early									
Plains	7250	1.45	1.67	0.73	0	19	1.50	2.75	6.0
Calif. Mariout	1455	1.49	1.65	1.33	1	16	2.00	2.25	7.0
Roho	3573	1.77	2.19	1.56	2	36	3.00	3.50	6.6
Late									
Montcalm	7149	0.67	1.05	0.35	0	0	1.75	0.63	7.5
Plush	6093	0.72	1.16	0.21	0	0	4.13	1.25	8.5
O.A.C. 21	1470	0.72	1.14	0.52	0	2	2.63	0.25	7.6

only slightly heavier than those of early varieties. The weight of roots on plants with a mild attack of root rot was less than that of healthy plants under the long photoperiod but not under the short photoperiod. Diseased root systems were coarser, consisting of more large coronal roots denuded of fine rootlets. Weight probably gives a poor indication of absorptive capacity.

Experiment 2 and 3.—In these experiments the leaves of plants grown in the 9-hour photoperiod were darker green, longer, and wider than those in the 15-hour photoperiod. Varieties differed as to leaf number under the 15-hour photoperiod (Table 2). The first leaves of Roho under the 15-hour photoperiod were long, but the newer leaves formed were increasingly shorter and narrower until the youngest ones were very small. Some varieties, such as O.A.C. 21, formed large leaves under these conditions. Tillers formed in the shorter photoperiod were more robust. Plains and Atsel tillered more at 15 hours than at 9 hours. Roho, California Mariout, and Vaughn tillered well under both photoperiods, whereas the remainder tillered more abundantly in the 9-hour photoperiod (Table 2). Culms of Peatland, Plush, Montcalm, and Vantage elongated well with the long light period, and poorly with the short exposure, while the reverse tendency was observed in Roho, California Mariout, and Atsel. Elongation of the culms in Roho was so restricted by the 15-hour photoperiod that not 1 of 36 heads was exserted from the boot (Fig. 3). As in experiment 1, all varieties developed more rapidly in the longer photoperiod and the varieties differed in ratio of dry weights of shoots at the 2 photoperiods.

In general, the total mass of roots was less with the long photoperiod than with the short photoperiod, but that of Montcalm was nearly the same under both light treatments. Root weights were reduced by the disease in both light treatments (Fig. 4). The barley root systems of fall-winter experiments were coarser than those of winter-spring experiments, in these and other experiments. Hoagland and Broyer (7) observed

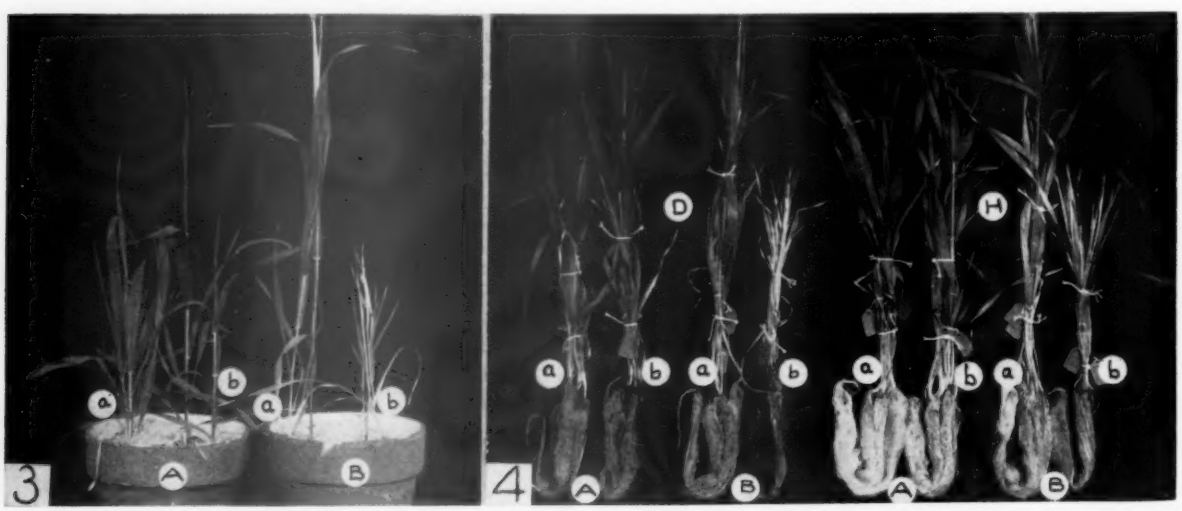

FIG. 3–4. Fig. 3. Barley plants grown under 9-hour day (A) and 15-hour day (B). O.A.C. 21 barley (a) and Roho barley (b). Fig. 4. Barley tops and roots grown under 9-hour daily photoperiod (A), and 15-hour (B); diseased plants (D), healthy plants (H). O.A.C. 21 barley (a) and Roho barley (b). The later variety does not tolerate a 15-hour photoperiod.

TABLE 3.—*May/June rainfall gradients illustrating marked variations between adjoining counties in South Dakota*[a]

County	Town	Precipitation, in. May	Precipitation, in. June	May/June per cent	Approximate distance, miles
Brookings	Brookings	2.82	3.80	74	31
Hamlin	Castlewood	2.64	4.00	66	
BonHomme	Tyndall	3.92	3.26	120	70
Charles Mix	Academy	2.98	3.79	79	
Edmunds	Ipswich	3.11	3.30	94	37
McPherson	Eureka	2.24	3.08	73	
Haakon	Hilland	2.85	2.96	96	55
Ziebach	Dupree	1.87	3.12	60	
Jackson	Cottonwood	2.66	2.50	106	24
Jackson	Kadoka	3.25	2.48	131	

[a] Climate and Man. U.S.D.A. Yearbook, 1941.

similar qualitative differences and attributed them to varying quality and intensity of sunlight.

Varietal performance in the root rot nursery was correlated with time of maturity. Yields and maturity rate of 16 varieties in the root rot nursery were compared with weights of roots and shoots of the same varieties in the greenhouse. Maturity class in the field was correlated with yield ($r = -0.80$). Dry weights of roots ($r = 0.66$) and shoots ($r = 0.51$) in the 15-hour photoperiod inoculated series were correlated significantly with yields in the root rot nursery. Relative maturity class in the field was correlated with root weight in the 15-hour photoperiod; (inoculated: $r = -0.67$, and check: $r = -0.52$). Data on check plants of the 15-hour photoperiod or either check or inoculated plants of the 9-hour day showed no correlation with field yields. Besert 13 responded well to long days in the greenhouse, but was very early, short, and a poor yielder in the field. Vantage grew well in all greenhouse tests but was relatively poor in the field.

The correlation of field yields with root weight in the 15-hour-inoculated greenhouse series indicated that root rot reactions of several varieties were revealed only under proper photoperiods, and that the disease was a factor in determining varietal ranking in the field. Tolerance to Pythium root rot appears to be associated with the rate of growth and development of the barley plant, which is modified by photoperiod.

DISCUSSION.—Climatic adaptation of a variety is largely the result of the ability of a plant to adjust to the concurrent sequence of rise and fall of temperatures, moisture levels, and light conditions, or the fit of the rhythm of climatic changes to vegetative development.[4] In South Dakota the rise in temperature from seeding to harvest is reasonably predictable, and photoperiod conditions fixed. Moisture remains as a great climatic variable. The ecological studies of Klages (8, 9) in this region pictured the climatic gradient from the relatively humid east to the drier west. The best varieties for the various areas reflected a slight change toward earlier varieties to as far west as central South Dakota, with a rapid change toward earliness in the western parts. In general, most observations emphasize the importance of timing vegetative development with moisture conditions. Grafius and Dirks (5) recently devised a scheme of estimating best maturity classes on this basis.

In an attempt to examine the role of the moisture factor in varietal adaptation more closely, the May-June rainfall of various areas was compared. Sizeable differences in precipitation occur in this critical period within and between regions of the state (Table 3). A rainfall in May approaching that of June would call for earliness, a fact reflected in the success of White Smyrna, Spartan, and Plains in some areas of western South Dakota. Varieties must be able to tolerate large fluctuations in moisture throughout their development to be adapted to much of this area, and the fact that a few varieties account for the bulk of the barley grown in most of the state indicates that these successful barleys make these adjustments. The failure of rainfall distribution to play as dominating a role as might be supposed in barley adaptation is illustrated by the fact that Tregal and Trebi are high in yield in all the major regions of South Dakota. Brookings, Huron, Highmore, and Pierre are located on a line running east to west in the center of the state. The May/June rainfall makes a gradient of 74 per cent, 79 per cent, 89 per cent, and 101 per cent indicating a need for increased earliness from east to west. Trebi and Odessa were selected (5) as representatives of the optimum maturity class of Highmore. These same barleys are high yielders at Brookings, 150 miles to the east and differing in proportion of May/June rainfall by 15 per cent, and Odessa is the long time standard variety of that area. The distribution of commercial varieties does not readily reflect small gradations in rainfall, either in quantity or time of fall. Apparently factors other than rainfall are active.

Edaphic factors play a strong role in plant distribution in areas of minimum rainfall (10, p. 345). Experi-

[4] Scharfetter, R. 1923. Phenology and Agriculture. Int. Rev. Sci. and Pract. Agr. 1: 561-572, cited from Klages, K.H.W. 1942. Ecological Crop Geography, p. 127. Macmillan, New York.

ments with partial sterilization of field soil at Highmore (unpublished) and in the root rot nursery (1) gave increases of 300 per cent in durum wheat and barley. These increases were obtained with no supplemental water. Much of the soil moisture was not available to the plants growing on untreated soil because of poorly functioning root systems. Differences in the extent and health of roots under these conditions can influence the performance of varieties.

The constancy and strength of the relative performance of varieties, especially when compared by maturity classes, on the root rot nursery illustrates the influence of a soil condition in adaptation. As conditions become more severe on this soil, as reflected in lower mean yields, the calculated optimum maturity class shifted toward lateness, the reverse of the expected. The existence of compensatory systems may explain this apparently irrational feature of adaptation (11, p. 120). Climatic features favor earliness in much of central and western South Dakota, but the associated weakness of the early classes in respect to root rot may partially cancel out their advantage. The problem of bringing barley in harmony with this environment is thus complicated by (at least 2) opposing factors.

Farmers and agronomists have long recognized that barley is more sensitive to most soil troubles than corn, oats, or wheat (6, 8). Barley yields were more closely correlated with rainfall than those of the other crops studied by Weaver in South Dakota (13). Among the major cereals, barley responded most to phosphorus.[5] Barley usually develops at a more rapid rate than corn, oats, or wheat. This rapidity of development may place a premium upon an adequate and constant supply of the essentials for growth. Selection of the earliest varieties may serve to accentuate these deficiencies.

Calculations of grain produced per day, from planting to harvest, revealed a definite weakness in the early groups. A possible explanation may lie in competition for food materials within the plant. Early barleys enter the reproductive phase rapidly, or the vegetative phase is quickly subordinated. Energy required to remove water from the soil rapidly increases as the soil dries (12). Capillary movement is soon insufficient to supply the needs of the plant, and an actual extension of the root system to tap fresh soil moisture is necessary (2, p. 149). Destruction of roots by a disease aggravates the difficulty. When the leaf area is developing, the energy need of the root system is great, especially in relatively dry soils. In contrast, excessive tiller and leaf development during favorable growing conditions early in the season places greater stress on these plants later in the season. Later maturing varieties may maintain a better balance between vegetative and reproductive activities and would consequently be better able to cope with the above sequence. The decreased size of the last formed leaves of Roho barley in the long-light day (Fig. 3) as compared to those of O.A.C. 21 is evidence of curtailed vegetative activity, as well as the inability to project the heads from the boot.

There is an extensive literature on Pythium root rot as it occurs on the cereals and grasses in the northern prairies and plains in areas of spring culture, but a limited or nonexistent literature on the same disease as it occurs in the winter cereal areas of the southern plains and Southwest. Possibly this distribution of the literature offers circumstancial evidence on the distribution and importance of the disease and supports the maturity rate and root rot relations. Winter cereals have an extended period for the development of the vegetative phase and an earlier more favorable environment for grain production than frequently occurs in the spring grain areas. Under this environment the plants are not subjected so frequently to the energy stresses of the more rapidly developing spring cereals, particularly in northern climates where the advance of day length and temperatures from spring to summer proceeds at an accelerated rate. Both the attack of Pythium root rot and the effect of the depleted root system upon plant production may be influenced greatly by the rate of change in environmental sequences in the 2 areas.

The shift of desirable maturity class toward lateness under severe root rot conditions indicates that varieties on the root rot nursery may be later than the optimum class for more favored soils, and that barleys selected under high fertility may be too early for best growth on less favored sites. This discussion is based upon classes, or populations, not individuals. Good varieties are outstanding individuals and may not represent class relationships. It is not meant to imply that a good early variety is an impossibility, but rather, that the medium-late varieties have a greater chance of success where Pythium root rot is a factor. Extremely late barleys, although generally not damaged severely by this disease, are more subject to the hazards of drought, rust, hot winds, insects, and hail than the earlier. Knowledge at present is not adequate to explain barley adaptation in physical terms. The best varieties may not fit a single major factor best, but they may strike a tolerable medium with the many factors of the environment. Dobzhansky (3, p. 216) has stated the principal in general terms in the following quotation: "The evolutionary implications of nature's annually recurrent drama of life, death, and resurrection have not been sufficiently appreciated. In order to survive and reproduce, any species must be at least tolerably well adapted to every one of the environments which it regularly meets. . . . Faced with the need of being adapted to diverse environments, the organism may be unable to obtain maximum efficiency in any one of them. Changeable environments puts the highest premium on versatility rather than on perfection in adaptation."

The fluctuating environmental conditions of the western prairies and plains pose a tremendous problem to the plant breeder—not one of fitting a fixed and pre-

[5] Unpublished data, courtesy of L. O. Fine, Agronomy Department, South Dakota Agricultural Experiment Station.

dictable sequence, but one of producing a flexible plant. Sources of "flexibility" in barleys may be discovered by physiological investigations designed to resolve barleys into their major ecotypes. Trebi is an example of flexibility; it has produced good crops from the prairies of Canada southward and is successful when fall sown in parts of the Southwest. It resembles the Coast type morphologically, but is not fixed to the Coast-type climate. The present observations indicate that most of the Egyptian barleys, in contrast, are rigidly adapted to the Coast-type climate, probably through strong response to photoperiod as in California Mariout and Roho. Resolution of the greater groupings of barley into similar physiological units would be of major assistance in solving problems of adaptation including disease reaction.

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INHERITANCE OF RESISTANCE TO TOMATO RACE O OF PHYTOPHTHORA INFESTANS[1]

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SUMMARY

Two types of resistance to the common tomato race of *P. infestans* were found. The first type, controlled by a dominant gene (or genes) and inherited in a simple Mendelian fashion, was present in West Virginia accessions 36 and 106. Plants with this gene formed small, necrotic, nonspreading lesions. The second type of resistance is controlled by multiple or minor genes. Plants with a large number of these genes formed small, atypical, sparsely sporulating lesions approximately ½ in. in diameter when inoculated at 4-6 weeks of age. When inoculated at 2-3 weeks of age, these multiple-gene plants were killed by the common tomato race. Multiple-gene resistance was found, in varying degrees, in West Virginia accessions 19 and 731-1, and in the varieties Southland and Wisconsin "55". The dominant genotypes were susceptible to a specialized tomato race. West Virginia tomato accessions 700 and 915 were found to be resistant to the specialized race. One preliminary test of the F_2 of Marglobe × 700 suggested that another dominant gene (or genes) for resistance may be present in 700. The single dominant gene in accession 106 was designated as TR_1. The common tomato race was labeled *tomato race 0* (T_0) and the specialized race attacking TR_1 genotypes was labeled *tomato race 1* (T_1).

It was not until after the epiphytotic of 1946 that serious attention was given to a search for resistance to tomato late blight. In 1946, certain commercial varieties were recognized as having a degree of resistance to *Phytophthora infestans* (Mont.) de Bary in some localities but were susceptible in others (2). Richards and Barratt (12) screened 137 accessions of *Lycopersicon* for resistance to late blight and concluded that although differences in resistance may exist within the genus, they were not discernible in their artificial tests. Andrus (1), however, classed 17 varieties as resistant in the 1946 epiphytotic, but noted that there

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were differences in degree of resistance within these varieties.

In 1952, Bonde and Murphy (5) reported that resistance to tomato late blight was present in the variety Danmark and in Red Cherry, a small-fruited variety. The resistance in Red Cherry appeared to be dominant since F_1 plants from crosses of Red Cherry with susceptible varieties were resistant. The resistance in Danmark appeared to be of a different type since the F_1 plants showed partial to total susceptibility. Also, in 1952 Walter and Conover (13) observed that 2 small-fruited tomato accessions used as ornamentals had a high degree of resistance to late blight when grown in southern Florida. F_1 plants of resistant × susceptible crosses were susceptible. The mode of inheritance was not described, but the reactions of the F_2 and F_3 populations suggested to them that resistance was due to 1 main factor, affected by 1 or more modifying factors. Ferguson, et al (7) reported resistance in certain accessions from the Philippine Islands but did not study the mode of inheritance. Other sources of resistance were listed by Gallegly (9) and Gallegly, et al (10).

The fact that a variety may show resistance in one locality and not in another, or that it may be resistant one year and susceptible another, was explained, in part, when Gallegly (8) discovered the existence of physiologic tomato races of *P. infestans*. One race was pathogenic to the commercial variety Break-O-Day but not to accessions 19, 731, 36 and 106; a second race attacked the commercial variety, 19, and 731 but not 36 and 106; still a third race was pathogenic to all tomato accessions. After studying the reactions of 45 isolates of the pathogen on Rutgers, Marglobe, Wisconsin "55", 19, 36, and 106, Wilson and Gallegly (14) concluded that the first 2 races originally recognized by Gallegly differed mainly in degree of virulence. Isolates pathogenic to 19 were more pathogenic to Marglobe and Rutgers than were isolates nonpathogenic to 19. Thus, on these tomato hosts, 2 tomato races were recognized among the 45 isolates; one was pathogenic to Marglobe and Rutgers but not to 36 and 106 and the other was pathogenic to all the tomato hosts. The first race may be considered the common tomato race and the second a specialized tomato race. An example of the common race would be Gallegly's M-7 isolate; an example of the specialized race would be his M-13 isolate (8). Conover and Walter (6) also reported the occurrence of physiologic tomato races when they noted in their 1952 plots that a virulent race of *P. infestans* was destructive to their P-1 source of resistance.

It is evident that there is some resistance to late blight in tomato and that there are races of the pathogen, but more information is necessary for an effective program of breeding tomato for resistance to the disease. This work was undertaken to learn more about the nature and extent of resistance to late blight in tomato and to study the mode of inheritance of resistance to the M-7 isolate of the common tomato race in crosses between 3 resistant accessions and certain susceptible commercial varieties. A limited study was also made of resistance to the M-13 isolate of the specialized tomato race.

MATERIALS AND METHODS.—West Virginia accessions 19 and 731 were obtained from Conover and Walter; 19 is the same as their P-1 accession and 731 is one of their breeding lines with P-1 resistance. Accession 36 was obtained from D. E. Ellis and W. S. Barham of North Carolina State College, Raleigh, North Carolina, who observed that it was resistant in their field tests; accession 106 is the same as PI 108245. Accession 19 has small pear-shaped fruit whereas 36 and 106 have cherry-sized fruit. The fruit of 731 approaches commercial size and shape.

Crosses were made in the greenhouse during the winter and spring months of 1951 through 1953. Single-plant selections were made from both greenhouse and field grown plants. Seeds were planted thickly in rows and the seedlings transplanted to flats before testing for resistance or the seeds were planted thinly and the plants left undisturbed. Plants were 4-6 weeks old when inoculated.

The M-7 isolate of *P. infestans*, used in all tests for segregation, was grown in test tubes at 20°C on lima bean agar (frozen lima beans, 250 g; agar, 15 g; distilled water to make 1000 ml). This isolate has both potato and tomato race characteristics and is classed as *potato race 0* and the *common tomato race* (8, 14). Inoculum was prepared by washing sporangia from the surface of agar slants (7-21 days old) and allowing the suspension to incubate at 10-12°C for 2 hours to induce liberation of zoospores.

The plants were placed in constant-temperature humidity rooms (20°C, 100 per cent relative humidity) for a few hours before inoculation and left in the rooms for 12-24 hours after inoculation before removing to a greenhouse at 20°C. After 7-10 days the plants were examined and placed in one of the following disease index classes unless otherwise specified: 1) Immune; no evidence of infection; 2) Resistant; necrotic flecks and small lesions; 3) Moderately resistant; lesions larger; may spread slowly; 4) Susceptible; severe and active stem and leaf lesions; plants not dead; 5) Very susceptible; plants dead or dying. The plants in classes 1, 2, and 3 were considered resistant whereas those in 4 and 5 were considered susceptible. The *chi square* method was used to test for goodness of fit of the data to simple Mendelian ratios. The approximate probability value for each *chi square* was interpolated from a logarithmic distribution curve.

EXPERIMENTAL RESULTS.—During routine inoculations to identify the race characteristic of many isolates of *P. infestans*, it was observed that West Virginia accessions 36 and 106 gave similar reactions (14); when resistant both would react by forming specks and small nonspreading lesions. These 2 accessions have continued to show a high degree of resistance to the M-7 isolate even though the conditions of each experiment varied. Accessions 19 and 731-1 likewise gave similar reactions to individual isolates of the

pathogen. However, the reactions of 19 and 731-1 often varied with the conditions of the test. Exact reasons for the variation have not been determined but age of plant and age and concentration of inoculum are involved. It is likely that environmental factors also influence the reactions. On older plants of accessions 19 and 731-1 (approximately 6 weeks), lesions are less numerous than on susceptible varieties and usually cease spreading after reaching a diameter of approximately ½ in. Stem lesions do not girdle the plant at this age but may elongate. When younger plants of accessions 19 and 731-1 are inoculated, however, the growing points of some may be invaded and the plants killed. Two- to 3-week old seedlings of these 2 accessions may show 100 per cent mortality when inoculated with a heavy concentration of zoospores of the M-7 isolate.

Reaction of breeding progeny from accessions 36 and 106.—Accession 106 and 2 single plant selections of accession 36 were crossed with varieties Wisconsin "55", Southland, Marglobe, and Rutgers.

The F_1 of these crosses (Table 1) were resistant. A few plants of varieties Wisconsin "55" and Southland were placed in the resistant class; all Rutgers and Marglobe plants were classed as susceptible. The F_2 generations (Table 1) segregated in the ratio of 3 resistant to 1 susceptible. Only the F_2 plants from cross X9 (Rutgers × 106) failed to fit the 3:1 ratio but instead segregated 2:1. Since the plant numbers of this cross were small and since all other F_2 plants of crosses with accession 106 segregated in the ratio of 3:1, attempts to explain the 2:1 segregation are not made. Again Wisconsin "55" and Southland were less susceptible than Rutgers and Marglobe but only 2 plants of Wisconsin "55" were classed as resistant. Three of 5 F_2 selections of X1 were homozygous for resistance and all 5 of the X7 selections were heterozygous and segregated in the ratio of 3:1. Thus 3 of the 10 F_2 selections were homozygous whereas 7 were heterozygous for resistance. Since susceptible plants were eliminated in the F_2 generation, no homozygous susceptible individuals were expected here.

Ratios of segregation in the first and second backcross progenies (14 different back-crosses) were, in all cases, near 1:1. Selfed resistant selections from backcrosses segregated in a 3:1 ratio; a number of homozygous resistant lines were selected from the third generation, some with 36-resistance and some with 106-resistance.

The genes for resistance in accessions 36 and 106 appear to be identical since both have given similar reactions under different conditions to a number of different isolates (14). Plants from X38, a cross between 2 heterozygous lines, one having 36 and the other 106 as sources of resistance (X27A-1 × X30A-2-2), segregated 30 resistant and 14 susceptible (P value 3:1 = 0.30). This 3:1 segregation ratio supported, but did not establish, the hypothesis that the genes were the same; further studies of progenies from crosses involving the 2 sources of resistance are necessary to decide this question.

Reaction of progeny when 731-1 was used as the source of resistance.—The progeny from crosses of accession 731-1 with the commercial varieties Marglobe and Southland were inoculated with the M-7 isolate in an effort to determine the mode of inheritance of the resistance carried by 731-1. Although some F_2 plants were resistant and some had been killed, there was a gradation in degree of susceptibility or resistance between these 2 extremes. Individual plants could be placed in 1 of 4 disease index classes (Table 2). All Marglobe plants were placed in classes 4 or 5 while a small percentage of Southland plants were placed in classes 3 and 3+. A high percentage of the plants of accession 731-1, however, fell into classes 3 and 3+. Seventy-eight per cent of the F_1 plants from the X11

TABLE 1.—*Resistant and susceptible F_1 and F_2 plants from crosses of accession 36 and 106 × commercial varieties when inoculated with the M-7 isolate of P. infestans*

Cross Number and Parent	Parentage	F_1		F_2		
		Resistant	Susceptible	Resistant	Susceptible	P[a] 3:1
		No.	No.	No.	No.	
X1	Wis. "55" × Ac. 36-1	26	0	113	46	0.24
X2	Marglobe × Ac. 36-1	54	0	44	17	0.59
X3	Wis. "55" × Ac. 36-4	28	0	45	16	0.82
X4	Marglobe × Ac. 36-4	45	0	52	18	0.96
X5	Rutgers × Ac. 36-4	7	0	31	10	0.91
X6	Southland × Ac. 36-4	16	0	38	12	0.87
X7	Marglobe × Ac. 106	15	0	98	33	0.96
X8	Wis. "55" × Ac. 106	18	0	44	18	0.47
X9	Rutgers × Ac. 106	20	0	40	24	0.02
X10	Southland × Ac. 106	19	0	43	14	0.94
Ac. 36-1		27	0	18	0	----
Ac. 36-4		19	0	18	0	----
Ac. 106		26	0	18	0	----
Wis. "55"		3	15	2	17	----
Southland		3	10	0	7	----
Rutgers		0	21	0	18	----
Marglobe		0	18	0	9	----

[a] Calculated by χ^2 method; not corrected for small numbers.

TABLE 2.—*Reaction of parent, F_1 and F_2 plants from crosses of accession 731-1 × Marglobe and Southland when inoculated with the M-7 isolate of P. infestans; plants 4 weeks old*

Variety or Cross	Parentage	Number of Plants	Percentage of Plants in Disease Class[a] 3	3+	4	5
Marglobe		18	0	0	22	78
Southland		38	8	10	32	50
Ac. 731-1		28	46	36	11	7
X11 F_1	Marglobe × 731-1	18	0	22	39	39
X14 F_1	Southland × 731-1	36	28	33	22	17
X11-1 F_2	Marglobe × 731-1	204	10	24	30	36
X14-1 F_2	Southland × 731-1	106	15	60	19	6

[a] 3 = leaf lesions atypical but may spread slowly.
3+ = leaf lesions spread to stem but stem lesions small, linear and nongirdling.
4 = stem lesions large and spreading to girdle plant.
5 = plant dead.

(Marglobe × 731-1) cross were placed in classes 4 and 5; none were resistant enough to be placed in class 3. Only 39 per cent of the F_1 plants from the X14 cross (Southland × 731-1) were placed in classes 4 and 5, however, and 28 per cent were given class 3 ratings. When segregation of the larger number of F_2 plants was studied, 10 per cent of the X11 plants were placed in class 3, 24 per cent in class 3+, and 66 per cent in classes 4 and 5. Fifteen per cent of the X14 F_2 plants were placed in class 3, 60 per cent in class 3+, and 25 per cent in classes 4 and 5. Thus the X14 F_2 gave a higher percentage of plants in classes 3 and 3+ than did the F_2 of X11.

Several of the plants of X11-1 and X14-1 which were given class 3 ratings were selfed for a study of the resistance in the F_3 generation (Table 3). Again, Marglobe plants fell into classes 4 and 5 whereas the majority of the plants of both Southland and accession 731-1 fell into classes 3 and 3+. A higher percentage of the plants of Southland and 731-1 were placed in the 3 and 3+ classes in this test than in the previous experiment (Table 2). In the previous test the plants were 4 weeks old when inoculated whereas in this experiment they were 6 weeks old. Although the number of plants was smaller in this test than in the earlier one, the F_2 of X11 and X14 also gave a higher percentage of plants in the 3 and 3+ classes. Even though there was a high degree of resistance in the F_3 of the X11 and X14 selections (72 to 93 per cent), a few susceptible individuals were found in all populations.

Thus it is apparent that resistance in accession 731-1 is controlled by multiple genes and that some of these genes are present in the Southland variety.

Reaction of certain commercial varieties and selection to the M-7 isolate.—As noted in some of the preceding tables, a few plants of the varieties Wisconsin "55" and Southland often were placed in the resistant class 3. It was shown in the preceding section that the Southland variety contained some multiple-gene resistance since crosses of 731-1 with it gave a higher percentage of resistant individuals in the F_1 and F_2 generations than did crosses of 731-1 with Marglobe. It was suspected, then, that the variety Wisconsin "55" also carried some of the multiple genes for resistance. One plant from the Valiant variety and 2 from Wisconsin "55" that were given class 3 ratings were selfed to determine whether resistance could be built up in these varieties by selection. The reactions of the selections to the M-7 isolate (Table 4) show that the

TABLE 3.—*Reaction of F_3 plants from resistant F_2 selections when inoculated with the M-7 isolate of P. infestans; plants 6 weeks old*

Variety or Cross	Parentage	Number of Plants	Percentage of Plants in Disease Class[a] 3	3+	4	5
X11-1	Marglobe × 731-1 F_2	19	52	11	21	16
X14-1	Southland × 731-1 F_2	20	65	15	15	5
X11-1-1	Marglobe × 731-1 F_2	60	80	13	7	0
X11-1-2	Marglobe × 731-1 F_2	54	48	39	9	4
X11-1-3	Marglobe × 731-1 F_2	46	46	33	17	4
X11-1-4	Marglobe × 731-1 F_2	39	62	26	10	2
X14-1-1	Southland × 731-1 F_2	95	76	17	4	3
X14-1-2	Souhtland × 731-1 F_2	87	57	26	8	9
X14-1-3	Southland × 731-1 F_2	62	62	24	14	0
Ac. 731-1		38	47	45	8	0
Southland		18	33	39	28	0
Marglobe		40	0	0	32	68

[a] 3 = leaf lesions atypical but may spread slowly—no stem lesions.
3+ = leaf lesions spread to stem—stem lesion small and linear.
4 = stem lesions larger and spreading to girdle plant—most leaves dead.
5 = plant dead.

TABLE 4.—*Reaction of certain tomato varieties and selections to the M-7 isolate of P. infestans; plants 6 weeks old*

Variety	Number of Plants	Percentage of Plants in Disease Index Class[a] 3	3+	4	5
Marglobe	40	0	0	32	68
Rutgers	44	0	9	27	64
Ac. 36-1	9[b]	0	0	0	0
Ac. 19	22	10	45	45	0
Ac. 731-1	38	47	45	8	0
Valiant	35	11	3	26	60
Valiant-1	57	7	2	30	61
Wis. "55"	74	19	32	35	14
Wis. "55"-1	87	23	35	32	10
Wis. "55"-3	58	30	44	17	9
Southland	18	33	39	28	0

[a] 3 = leaf lesions atypical but may spread slowly—no stem lesions.
3+ = leaf lesions spread to stem—stem lesions small and linear.
4 = stem lesions larger and spreading to girdle plant—most leaves dead.
5 = plant dead.
[b] All individuals were placed in disease index class 2.

varieties Wisconsin "55" and Southland have a moderate degree of resistance when compared with Marglobe and Rutgers varieties. A majority of the plants of the former 2 varieties were placed in classes 3 and 3+ whereas a majority of those of the latter 2 varieties were placed in classes 4 and 5. This resistance was not so high as that shown by 36-1 (dominant-gene accession), which formed only small necrotic spots. A low level of resistance was also noted in Valiant. The single-plant selection of Valiant did not increase the degree of resistance. The 2 single-plant selections of Wisconsin "55" showed a slightly higher degree of resistance than did the original unselected variety. Accession 731-1 had a higher level of resistance than did 19 (Florida P-1) even though the resistance carried by 731-1 was obtained from 19. A similar situation may be noted in Table 3 where resistant F_2 selections from crosses of 731-1 with Marglobe and Southland showed higher degrees of resistance in the F_3 than did their resistant parent 731-1.

Resistance to the M-13 isolate of P. infestans.—Since West Virginia accessions 36 and 106 were susceptible to a specialized race of the late blight pathogen (8), a search for additional sources of resistance was initiated. As a result of this search (10) it was found that West Virginia accession 700 (PI 204996), when 6 weeks old, was resistant to this race. In a more recent test it was found that West Virginia accession 915 also was resistant to the specialized race. In the same test 6-week old plants of accession 36-1 were less susceptible to this race than were plants of accession 106. The plants of accession 36-1 reacted to the specialized race as Southland plants did to the common race; the plants of accession 106 were killed by the specialized race. Accessions 700 and 915 were obtained through the courtesy of J. S. Niederhauser and R. W. Richardson of the Rockefeller Foundation, Mexico. In one test of the F_1 and F_2 plants of a Marglobe $\times$ 700 cross with the common tomato race (M-7 isolate), the first generation individuals were resistant and the second generation segregated 68 resistant and 4 susceptible, approximately a 15:1 ratio. This indicated that there may be duplicate dominant genes in accession 700. Further tests of the progeny for susceptibility to the specialized race of the pathogen are necessary to determine the type of resistance carried by accession 700.

DISCUSSION.—There are 2 types of resistance to tomato late blight. The first type, found in West Virginia accessions 36 and 106, is controlled by a dominant gene (or genes) inherited in a simple Mendelian fashion. Since accessions 36 and 106 gave similar reactions to a number of different isolates of *P. infestans* it is thought that the dominant genes carried by the 2 tomato accessions are the same. Susceptible individuals in progeny from 36 were not so severely diseased as were susceptible individuals in progeny from 106, suggesting that 36 carried factors for resistance other than the single dominant gene. The dominant gene resistance carried by 36 and 106 appears to be similar to that found by Bonde and Murphy (5) in their Red Cherry tomato. It is not known whether the resistance gene in 36 or 106 is the same or different from that in Red Cherry.

The second type of resistance is controlled by multiple genes. These genes were present in accession 731-1. The F_1 plants of Marglobe $\times$ 731-1 were intermediately resistant or susceptible whereas those from Southland $\times$ 731-1 gave some resistant, some intermediate, and some susceptible individuals. A gradation from resistance to complete susceptibility was noted in the F_2 progeny, indicating that each individual had a different number of genes for resistance. When divided into 4 classes, the F_2 plants for the Marglobe $\times$ 731-1 cross showed 34 per cent resistant (disease indices 3 and 3+) and 66 per cent susceptible (diseases indices of 4 and 5) whereas those of Southland $\times$ 731-1 showed 75 per cent resistant and 25 per cent susceptible. Selections of resistant F_2 plants from both crosses proved to be 72-93 per cent resistant in the F_3 generation.

The F_2 segregation of the cross of 731-1 with Southland suggests that resistance is due to a single dominant gene whereas that of the cross with Marglobe suggests that resistance is controlled by a single recessive gene. If resistance of the Southland $\times$ 731-1 cross is controlled by a single dominant gene then the F_1 should have shown 100 per cent resistance. However 61 per cent were resistant and 39 per cent susceptible. If resistance of the Marglobe $\times$ 731-1 cross is recessive then all the F_1 plants should have been susceptible; however, 22 per cent were placed in the resistant 3+ class. It is concluded, therefore, that the resistance carried by accession 731-1 is controlled by multiple genes, and that the Southland variety carries some of these genes. The variety Marglobe appears to carry few or none of them.

If the parents in these crosses were homozygous for multiple-gene resistance or for susceptibility, one would expect all the F_1 individuals to give the same disease index reading. However, both the parents and F_1 reacted as heterozygous lines. It has been observed repeatedly that plant age has an influence on the degree of resistance exhibited by the multiple-gene resistant lines. Young 3-week old seedlings of 731-1 and Southland are usually killed by the M-7 isolate whereas older plants are resistant. The influence of age on the degree of resistance shown by these 2 lines can be seen if their reactions in Tables 2 and 3 are compared. The 4-week old plants in Table 2 gave a higher percentage of susceptible plants than did the 6-week old plants in Table 3. Thus, a tomato variety homozygous for multiple-gene resistance would perhaps give a heterozygous reaction since it is likely that the individuals in a population differ in physiological age.

A moderate degree of resistance, approaching that of Southland, was noted in the commercial variety Wisconsin "55." A still lower degree of resistance was noted in Valiant. Since the reaction of these varieties was similar to, but lower than the resistance in 731-1,

it is thought that the resistance in these varieties is controlled by multiple genes. Plants of the Wisconsin "55" and Valiant varieties which showed resistance were selfed in an effort to build up resistance in these varieties. The Valiant selfed line showed no more resistance than did the original unselected variety. The 2 Wisconsin "55" selections showed a small increase in degree of resistance. These single-plant selections were inoculated when 6 weeks old; perhaps if the plants had been younger or the inoculum heavier, then more of a difference in degree of resistance between these selections and the original variety would have been observed. Further work with these and other varieties is necessary to determine whether multiple-gene resistance already present in commercial varieties can be built up by selection.

It is likely that the 17 varieties listed by Andrus (1) as resistant during the 1946 epidemic carry the multiple-gene type of resistance. Perhaps the variety Danmark, listed by Bonde and Murphy (5) also carries the multiple-gene resistance. The failure of Richards and Barratt (12) to observe resistance in some of these same varieties may be explained partially by the fact that under the conditions of their artificial tests multiple-gene resistance was not expressed. It is possible, too, that different physiologic races of the fungus were used by these workers.

Although Gallegly (8) indicated that 3 tomato races of *P. infestans* existed, Wilson and Gallegly (14) suggested that the original first and second races be considered as one. Isolates fitting the description of the original second race were found to differ from those of the first only in degree of virulence. Thus only 2 tomato races were recognized, one pathogenic to accessions with recessive genes but nonpathogenic to those with dominant genes, and the other pathogenic to both the recessive- and the dominant-gene accessions.

Plants with the single dominant gene, then, are resistant to the common tomato race but susceptible to the specialized tomato race. Those with multiple genes are resistant to the common race when 4-6 weeks old but may be susceptible when 2-3 weeks old. The younger seedlings may, however, be resistant to isolates of the common race low in virulence. It is likely that plants with multiple-gene resistance also would show resistance to some of the isolates of the specialized tomato race. The fact that plants of accession 36-1 showed a low degree of resistance to the specialized race when plants of accession 106 were killed, suggests that accession 36-1 carries multiple genes in addition to the dominant gene.

Resistance to the specialized race was found in accessions 700 and 915. The F_2 generation of Marglobe $\times$ 700 segregated in the ratio of 15 resistant and 1 susceptible to the common race indicating that duplicate dominant genes were present in the accession. The progeny have not been tested for resistance to the specialized race and as a result it is not known what type of resistance accession 700 carries; it is possible that only 1 dominant gene plus a large number of multiple genes are present.

The 2 types of resistance in tomato to the tomato races parallels the 2 types recognized in potato to potato races of the pathogen. Black (3) noted 4 dominant genes in *Solanum demissum* derivatives, which were inherited independently in a simple Mendelian fashion. They were called major genes. He also noted a series of minor genes, which controlled the degree of susceptibility in susceptible individuals and the degree of necrosis (resistant reaction) in resistant ones. The 4 dominant major genes for resistance to potato races of *P. infestans* were labeled R_1, R_2, R_3, and R_4. Each gene, when present, conferred resistance to the common potato race. When new specialized potato races of the fungus that attacked plants with 1 or more of the major genes were found, a system of nomenclature for designating potato races and genes controlling immunity from these races was proposed (4). The common race was labeled potato race 0; the race found to attack only recessive and R_1 genotypes was labeled race 1; the one found to attack recessive and R_2 plants was labeled race 2; etc. When a race was found to attack plants with more than 1 gene it was labeled as a combination race. For instance, race 1,2 would attack the recessive, R_1, R_2, and R_1R_2 genotypes. Similarly, potato races have been found to attack the other major gene combinations. Recently, potato race 1,2,3,4, which will attack plants with all 4 genes ($R_1R_2R_3R_4$ genotype), was found (11).

The tomato multiple-gene resistance is similar to the potato minor-gene type and the tomato dominant-gene resistance is similar to the potato dominant-gene type. However, only 1 dominant gene, with an indication of others, has been found to give resistance to tomato late blight. Since the dominant gene for resistance found in tomato is different from any now known in potato (14), it is suggested that it be designated TR_1. The prefix "T" serves to distinguish the tomato gene from the potato R_1 gene. It is further suggested that the TR_1 designation refer to the gene present in accession 106. If the dominant gene in accession 36 and the genes in accessions 700 and 915 prove to be different, then they should be given additional numbers, TR_2, TR_3, etc. For the present it is suggested that the commercial variety Rutgers be used as the recessive differential host and accession 106 (PI 108245) as the TR_1 host.

Since the dominant-gene resistance in tomato is similar to that in potato, and since a specialized race was found to attack plants with this gene, a system similar to the one used for designating potato races is suggested for designating the tomato race. Thus the common tomato race of *P. infestans* that attacks recessive tomato varieties is designated as *tomato race 0* and the specialized race that attacks varieties with the TR_1 gene is designated at *tomato race 1*.

Because of parallel systems and the possibility of confusion, Wilson and Gallegly (14) suggested that

the potato race number be prefixed with P and the tomato race number with T. This is necessary since 2 different isolates of the same tomato race may react as 2 entirely different potato races; likewise different isolates of the same potato race may react as different tomato races.

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COINED NAMES FOR FUNGICIDES

Common coined names have now been assigned to 10 fungicides, with the approval of the Interdepartmental Committee on Pest Control. The majority of these names had their origin in the Special Committee on Fungicide Nomenclature of the American Phytopathological Society; some of them were submitted by the manufacturer of the fungicide in question. Dr. Dwight Powell was chairman of the Special Committee on Fungicide Nomenclature from 1947 to December 1951; the writer was chairman from December 1951 to September 1954. The procedure for assigning names to pest control chemicals has been revised so that nomenclature is now under the jurisdiction of American Standards Association Sectional Committee on Common Names for Pest Control Chemicals K62. Dr. S. E. A. McCallan is the representative of the American Phytopathological Society on this committee. The list of coined names is as follows:

captan	*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
chloranil	tetrachloro-*p*-benzoquinone
dichlone	2,3-dichloro-1,4-naphthoquinone
ferbam	ferric dimethyldithiocarbamate
glyodin	2-heptadecyl-2-imidazoline acetate
maneb	manganous ethylenebis[dithiocarbamate]
nabam	disodium ethylenebis[dithiocarbamate]
thiram	bis(dimethylthiocarbamoyl) disulfide[1]
zineb	zinc ethylenebis[dithiocarbamate]
ziram	zinc dimethyldithiocarbamate

—GEORGE A. ZENTMYER, *University of California, Riverside, California.*

[1] Alternate chemical name: tetramethylthiuram disulfide.

FALL APPLICATION OF BORON SPRAYS AS A CONTROL FOR BLOSSOM BLAST AND TWIG DIEBACK OF PEARS [1]

Folke Johnson, D. F. Allmendinger, V. L. Miller, and Dorothy Polley [2]

SUMMARY

A blossom blast and twig dieback of pears occurs in southwestern Washington. The most noticeable symptom appears shortly after the trees reach full bloom when the blossoms begin to shrivel and die. The distal tips of the current season's growth abscise and on the remainder of the spur 2 or more laterals develop, producing a short, compact, stubby growth. Leaf development is retarded and in severe cases the young leaves fall off, resulting in a dieback of terminal branches. All trees in an orchard are not necessarily affected and only portions or all of a tree may show symptoms. Severity of the disease may vary in an orchard or in the same tree from season to season, depending upon climatic conditions. Susceptible varieties noted are Bartlett, Anjou, and Bosc.

The disorder was shown to be due to boron deficiency. Soil applications of boron gave erratic and inconclusive results. When this element was applied as a foliar spray (as Polybor 1:100) in June, some control was effected in one orchard, but similar applications were ineffective in another. When boron sprays (as borax 3:100 or Polybor 1 and 2:100) were applied in the fall, complete control was obtained in all trees in the 5 orchards studied.

Chemical analyses of samples from 4 orchards at 4 times during the growing season showed that the sprayed material was consistently higher in boron content than the diseased. The healthy unsprayed samples were intermediate between the 2. The differences in boron content between leaf samples were most apparent in early spring.

For 25 years or more, pear trees in southwestern Washington have been affected by a disease referred to as "blossom blast" and "twig dieback" (1). Areas of most extensive damage are in Clark, Skamania, and Klickitat counties where Bartlett and Anjou are the principal varieties. Orchards in these localities are nonirrigated and grow on a variety of soils ranging from sandy to clay loams. Precipitation is 20-40 in. a year, the period from May through September normally being very dry. The orchards are clean cultivated in summer and planted in fall to a cover crop of rye, or rye mixed with vetch, which is disced into the soil in early spring.

Severity of the disorder fluctuates from year to year and seems to depend on the amount of available moisture in the soil during the preceding growing season. When soil moisture is low, severe symptoms develop the ensuing spring.

From the beginning of these investigations, it was suspected that the disorder was in some way related to a nutrient deficiency as observed in British Columbia (11), California (7), and Oregon (10), but the fruit symptoms seen in Oregon are not apparent in Washington. In these areas, soil application of boron or injection of boron solution into the trees afforded control but numerous attempts to obtain correction of the disorder in southwestern Washington by these methods gave only erratic and inconclusive results. Therefore, the cause of the disease was sought among pathogenic organisms, but inoculations with hundreds of bacterial and fungus isolates from affected tissues failed to reproduce the characteristic symptoms. The present paper discusses the problem and its ultimate control with post harvest spray applications of boron.

SYMPTOMS.—The most noticeable symptom appears when the blossoms begin to shrivel and die. (Fig. 1, A). Such blossoms may or may not fall off. The distal tip of the current season's growth abscises, the remaining portion becomes enlarged (Fig. 1, B), and 2 or more laterals develop, resulting in a short, compact, stubby growth. Young spurs develop necrosis internally. Leaf development is retarded; in severe cases, the young leaves fall off and an entire branch dies back (Fig. 1, C). In such a branch there is pronounced necrosis in the phloem and cambial region (Fig. 1, D). Twigs that have not been killed subsequently develop leaves and by mid-summer look normal; thus it is sometimes difficult to diagnose accurately the condition late in the season. Leaves on affected twigs or branches are pale-green, narrow and cupped, sometimes having marginal scorch. These symptoms are generally more acute in the top than in the lower parts of the tree. On the trunks and scaffold branches appear numerous, sunken, cankers with a hard surface but which do not penetrate to the cambium; however, their relationship to boron deficiency has not been established.

Seldom do all trees in an orchard or all parts of a tree become affected, it not being unusual to see diseased and healthy specimens appearing adjacent to one another or to see a branch or 1 or more scaffold branches with symptoms. Moreover, the severity of the disease in the same tree may vary from one season to the next. The Bartlett and Anjou varieties are equally affected; while symptoms have also been observed in Bosc, the latter is not extensively grown in the area.

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[2] Plant Pathologist, Superintendent and Horticulturist, Associate Chemist, and Junior Chemist, respectively. State College of Washington, Western Washington Experiment Station, Puyallup, Washington. Work done jointly by the latter and the Southwestern Washington Experiment Station, Vancouver, Washington. Scientific Paper No. 1336, Washington Agricultural Experiment Stations, Pullman.

FIG. 1. A) Symptoms of blossom blast in Bartlett branches, healthy specimen at right. B) Twigs from boron deficient Anjou tree showing swollen apices and development of new laterals as a result of abscission of terminals. C) Anjou tree affected with blossom blast and showing dieback of top central branch. D) Branches from A, showing internal necrosis, healthy at right.

METHODS.—Most of the investigations were conducted in an orchard in Klickitat county, consisting of approximately 500 acres of Anjou with an additional 100 acres in Bartlett. Trees in the experimental block were more than 40 years old, except for a few replacements of different ages. When symptoms were most pronounced, each tree was given a "disease index" number. A tree with no apparent symptoms was scored "0," and as the disease syndrome became progressively more severe, intermediate numbers to "5" were given. The latter meant the tree was dead or dying.

EXPERIMENTAL RESULTS.—On October 16, 1951, 14 trees affected to varying degrees were sprayed with

borax at a concentration of 3 lb. in 100 gal. of water. At this date the leaves were still green. The following spring there was no evidence of the disease in any of the sprayed trees. All had a vigorous bloom, set a good crop of fruit, and bore foliage with a dark-green, healthy appearance. In contrast, some untreated trees on either side were diseased, with the usual variation in severity of symptoms.

The work was expanded greatly in 1952 when plots consisting of Bartlett and Anjou of various ages in 5 different orchards were established. A June spray was compared with one applied in October. Results in all 5 plots were the same; only those obtained in orchards 1 and 2 will be reported in detail.

Summer sprays.—Nine Anjou and 2 Bartlett trees in orchard 1, and 12 trees in each of 2 adjacent rows in orchard 2, were sprayed with Polybor 1:100 on June 24, 1952. The latter orchard consisted of 7-year-old Bartlett trees, which were severely affected.

The following spring, only 1 of the 11 sprayed trees in orchard 1 showed symptoms. All others in this block were healthy, while half of the 16 adjacent check trees were affected with disease indices from "1–3." In orchard 2, 23 of the 24 sprayed trees showed symptoms, with disease indices ranging from "1–5." In this case there was no improvement in the sprayed trees when compared with the checks.

Fall sprays.—On October 14, 1952, 12 trees in orchard 1 were sprayed with borax 3:100 and 16 with Polybor 2:100. On the following day in orchard 2, 34 trees were given a borax spray of 3:100, 31 received Polybor 1:100 and 18 Polybor 2:100. In each case at the latter location, a row of unsprayed check trees separated the treated rows. Disease index records were available for the sprayed trees before and after treatment in orchard 1. At orchard 2, no disease records had been kept previous to the spray applications in 1952; hence, only the 1953 disease index ratings are given. The number of trees which fell in each index group the year following the treatments were applied is given in Table 1. In no case did any sign of disease appear in any sprayed tree the year following the treatment. This fact was more striking in orchard 2 than in orchard 1. The sprayed rows of trees had a healthy bloom and foliage with a good set of fruit, while in the adjacent check rows the trees were severely affected (Fig. 2, A, B). In the remaining 3 orchards where 82 trees received boron sprays in the post harvest applications, all were completely healthy the following season of 1953, whereas adjacent check trees showed disease in various degrees of severity.

CHEMICAL ANALYSES. — Samples of bud clusters, leaves, and fruit were collected from 3 or more trees in each of 4 orchards at different times during the growing season of 1953 and analyzed for boron. The material was collected at random around the trees, usually to the height one could reach by standing on the ground but in some instances, where the material was scarce (as in certain disease specimens), the upper branches were also sampled. The leaves taken were mature spur leaves and included the petioles. Analyses for boron were by a modified procedure of the one described by Hatcher and Wilcox (8). These data are presented in Table 2.

According to the data, the developing bud clusters in early spring are high in boron content. Subsequent leaf analyses show that as growth continues, the boron content drops, then gradually increases during the greater part of the growing season. Samples from fall

TABLE 1.—*The effect of post harvest sprays with boron for control of blossom blast and twig dieback of pears. Applications made October 14-15, 1952*[a]

Location and treatment	No. of trees in each disease index group of: 0	1	2	3	4	5
Orchard 1						
12 trees sprayed with borax 3 lb./100 gal.						
Disease records before treatment, 1952	0	2	4	3	3	0
Disease records after treatment, 1953	12	0	0	0	0	0
16 trees sprayed with Polybor 2 lb./100 gal.						
Disease records before treatment, 1952	4	3	4	4	1	0
Disease records after treatment, 1953	16	0	0	0	0	0
54 adjacent check trees						
Disease records for 1952	10	11	17	8	7	1
Disease records for 1953	28	11	8	6	1	0
Orchard 2[b]						
34 trees sprayed with borax 3 lb./100 gal.						
Disease records after treatment, 1953	34	0	0	0	0	0
31 trees sprayed with Polybor 1 lb./100 gal.						
Disease records after treatment, 1953	31	0	0	0	0	0
18 trees sprayed with Polybor 2 lb./100 gal.						
Disease records after treatment, 1953	18	0	0	0	0	0
127 adjacent check trees						
Disease records for 1953	26	17	13	17	18	36

[a] In orchard 1, 17 of the trees were Bartlett and 65 were Anjou; in orchard 2, all trees were Bartlett.
[b] Disease index records unavailable for 1952.

FIG. 2. Control of blossom blast and twig dieback in Bartlett with fall application of boron spray. A) Row at left treated, right unsprayed. B) Close-up of sprayed and unsprayed trees from rows above. Pictures taken in August, 1953, following the previous October application.

sprayed trees contained the greatest amount of boron, diseased control the least, and healthy control somewhere intermediate between the two. The greatest difference in boron content between the diseased and healthy control and the boron sprayed material occurs in early spring.

DISCUSSION.—The boron available for the trees comes either from organic matter or from minerals in the soil. It is possible to have an adequate soil boron content and yet not have enough boron available for growth (9, 13). An important factor determining the amount of available boron in soils is the amount of moisture present (2, 4, 9). In the area studied, the growing season is generally dry and, hence, organic

TABLE 2.—*Analyses of boron from samples collected at different times during the growing season. Treated samples taken from trees sprayed the previous year*

Description of samples collected	Av. ppm B of samples collected in 1953 on: March 24	April 28	June 4	August 10
Diseased unsprayed control				
Bud clusters	18.			
Leaves		5.0	8.	19.
Fruit				12.
Healthy unsprayed control				
Bud clusters	38.			
Leaves		10.	16.	22.
Fruit			12.	17.
Boron spray — June application (Polybor 1 lb.: 100)				
Bud clusters	54.	—		
Leaves			18.	22.
Fruit			18.	13.
Boron spray—October application (Polybor 1 and 2 lb.: 100, and Borax 3 lb.: 100 combined)				
Bud clusters	56.			
Leaves		50.	26.	27.
Fruit			27.	19.

decomposition and mineral dissolution are retarded. This could explain why the deficiency is more severe following a dry summer than in seasons with adequate summer rains. It has been reported that, under boron deficiency conditions, root tips die sooner than stem tips (5). This effect would be important in the uptake of soil solutes even when rains occur in fall and winter.

The function of boron in plants has been discussed by Berger (3). It would appear that boron is a component of the cell wall and also necessary for cell division. In deficient plants the cambium and at least portions of the phloem disintegrate with an accumulation of carbohydrates in the leaves. It was also pointed out that boron is essential in metabolism and translocation of carbohydrates. More recently Gauch and Dugger (6) present evidence for the hypothesis that boron deficiency is in reality a sugar deficiency. They were able to show an increase of 50–85 per cent in the respiration of lima bean and pea root tips when grown in the presence of sucrose to which boron was added, in comparison with similar samples which had received no additional boron. From their interpretation the increased respiration resulted from the greater movement of sucrose "or its hydrolytic products." The function of boron in the movement of sugars and plant-growth-modifying substances has also been demonstrated by Mitchell et al (12).

In these studies, a post harvest spray was consistently better in controlling the deficiency than one applied in June. Better control by the summer application was obtained in orchard 1 where the Anjou variety predominated than in orchard 2 which consisted of Bartletts. This may be a varietal response, which the analyses did not detect under the present circumstances. More frequent sampling at regular intervals is necessary to clarify this point.

In the orchards studied it was shown that about 1 lb. of boron[3] to the acre applied as a spray was sufficient to give complete control. Thus for an outlay of about $0.50 in cost of material on an acre basis, an orchard can be brought into production the season following one with an onset of blossom blast and twig dieback.

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[3] Equivalent to 5 lb. of Polybor as used in these trials.

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NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

The opportunity for publicizing research in the field of plant pathology is available and should be utilized by our membership. The Public Relations Committee appointed Mr. Robert Rathbone, Information Specialist, as our public relations representative last March. Since then, Mr. Rathbone has prepared several excellent articles concerning research in plant pathology. Since we now have the vehicle for good public relations work, more source material is needed. If more of our Society members will assume the responsibility for sending copies of their papers to the Public Relations Committee at the time they are accepted for publication, the effectiveness of the Committee's activity will be greatly enhanced.

Canadian Pathologists were well represented on the program of the Second Annual Meeting of the Canadian Agricultural Chemicals Association held October 28 and 29, 1954, at Montebello, Quebec. Panel discussions were held on "Chemical Control of Plant Diseases in Canada" and "Some Results from the Canada Department of Agriculture Research program on pest control."

Plant pathologist, J. C. Carter, has been appointed Head of the Section of Applied Botany and Plant Pathology, Illinois Natural History Survey. Dr. Carter fills the vacancy left by the death of L. R. Tehon on October 17.

Forest disease and insect research was reviewed by specialists from the western United States, Canada, and Alaska in a conference in Berkeley, California, December 2-4, 1954. Seventy persons were present and participated. The program of the Disease Conference included a review of new studies at western research centers, disease research methods, and methods in forest disease surveys. One half day was devoted to joint sessions between entomologists and pathologists to discuss problems involving both forest insects and tree diseases. Dr. Vidar Nordin of the Calgary Forest Biology Laboratory, was elected Chairman of the Western Forest Disease Work Conference for 1955. Robert L. Furniss of the Pacific Northwest Forest and Range Experiment Station, Portland, Oregon, was re-elected Chairman of the Western Forest Insect Conference. The next meeting of these two work conferences will be held in Spokane, Washington, in November, 1955.

Phytopathologist E. E. Clayton, formerly in charge of tobacco disease investigations, U.S.D.A., has left the Department of Agriculture after nearly 25 years' service to accept the position of Director of Research for the American-Sumatra Tobacco Corporation. In his new capacity, Dr. Clayton will be located at Tallahassee, Florida, where he will continue his research on breeding for disease resistance in tobacco.

Membership in the Society as of August, 1954, totaled 1650. It consists of 125 Life Members, 15 Emeritus Members, 4 Life and Emeritus Members, 1489 Regular Members, and 17 Sustaining Associates.

Deaths: Bjarne Dundas, plant breeder with Associated Seed Growers, Inc., Milpitas, California, was killed in a highway accident in August, 1954. Mr. Dundas was known for his research work in California on resistance in beans to the different strains of powdery mildew and rust.

A. V. Osmun, Life Member, Emeritus Professor of Botany, University of Massachusetts, and former Head of the Department. Mr. Osmun conducted research on the ferns and flora of New England, on soil bacteria, and on shade tree diseases.

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